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Experimentelle Studien über die Funktion des reticuloendothelialen Systems

IV. Mitteilung
Bedeutung der Milz im Reticuloendothelialen System

Von

Shoichi Yamagata, Toshio Aratani, Kiyomi Miura und
(山形 敞一) (荒谷 利夫) (三浦 清美)

Kisao Otomo
(大友 喜佐雄)

Aus der Med. Klinik von Prof. Dr. T. Kurokawa, Tohoku Universität, Sendai

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Seit Aschoff[1] ist es allgemein anerkannt, dass die Milz ein wichtiges Organ des reticuloendothelialen Zellapparats ist. Aber Akazaki[2] betont, dass die Milzsinusendothelien zwischen den eigentlichen Endothelien und Reticuloendothelien stehen, weil sie sowohl funktionell als auch morphologisch als Reticuloendothelien nur dann wirken können, wenn sie besonders stark stimuliert werden.

Nach unserer früheren Mitteilung[3] kann der Kongorotindex die Funktion des RES und die Prothrombinzahl die Leberzellenfunktion verraten; deshalb wurde in der vorliegenden Untersuchung untersucht, wie die Milz auf die Funktion des RES und der Leber einwirkt. Die Auswahl der Versuchstiere sowie die Methodik war die gleiche wie bei der I. Mitteilung[4], und wir verglichen den Durchschnittswert von 2 oder 3 Kaninchen mit dem bei den Belastungsversuchen in der I. Mitteilung.[4]

Versuchsergebnisse

1. Einfluss der Milzexstirpation

Wie aus Tabelle I und II zu ersehen, bleiben der Kongorotindex und die Prothrombinzahl bei Laparotomieversuchen gegenüber den Kontrollversuchen fast unverändert, mit geringer Ausnahme des Kongorotindex für eine kurze Zeitspanne nach Laparotomieversuchen. Aber der Kongorotindex vermehrt sich bei der Milzexstirpation bis etwa zum 7. Tage, dann vermindert er sich und kehrt ungefähr nach 4 Wochen zum früheren Zustand wieder zurück. In diesem Falle ist eine Verminderung der Prothrombinzahl nicht so auffallend.

Wenn man 2 Leberlappen des Kaninchens am 18. Tage nach der

TABELLE I

Einfluss der Milzexstirpation für eine kurze Zeitspanne

		Nr. des Kaninchens	Vor. Op.	Zeit nach Operation (St.)					
				3	5	8	12	24	48
Kontrolle	K-index	1	62.6	66.3	66.7	69.4	64.5	58.8	65.8
		2	54.5	57.2	60.0	60.0	55.7	58.2	54.0
		3	37.5	49.9	56.6	50.0	40.7	44.3	54.8
		4	35.1	44.6	58.2	47.7	56.3	53.3	46.1
		Durchschn.	47.3	54.1	60.8	58.1	54.8	54.1	56.1
		Differenz		6.8	13.5	10.8	7.5	6.8	8.8
	P-zahl	1	1.00	1.03	1.03	1.01	0.98	1.01	0.98
		2	1.00	1.01	0.99	1.01	0.97	0.99	0.96
		3	1.00	0.95	0.98	1.01	1.01	1.01	0.95
		Durchschn.	1.00	1.00	1.00	1.01	0.99	1.00	0.96
		Differenz		0	0	0.01	-0.01	0	-0.04
Laparotomie	K-index	1	71.3	72.6	72.8	78.3	70.3	62.2	60.0
		2	50.0	53.5	65.0	53.7	64.4	52.7	55.2
		3	47.0	50.0	58.2	62.6	59.2	44.2	44.2
		Durchschn.	55.8	57.6	65.8	62.7	65.0	51.2	52.4
		Differenz		-5.0	-3.5	-3.0	1.7	-10.9	-12.2
	P-zahl	1	1.00	1.01	0.99	1.00	1.01	1.01	1.03
		2	1.00	1.02	1.00	1.02	1.02	1.01	1.00
		3	1.00	1.00	1.00	1.00	0.99	0.97	0.95
		Durchschn.	1.00	1.01	1.00	1.01	1.01	1.00	0.99
		Differenz		0.01	0	0	0.02	0	0.03
Milzexstirpation	K-index	1	55.2	61.8	84.1	82.0	82.7	51.7	57.8
		2	55.0	65.6	75.2	77.0	62.5	73.0	68.0
		Durchschn.	55.1	64.1	78.1	78.8	67.1	59.1	65.0
		Differenz		7.2	13.0	16.8	2.8	8.1	13.3
	P-zahl	1	1.00	1.00	0.99	1.01	1.02	1.04	1.05
		2	1.00	0.99	1.00	1.03	1.00	0.97	0.98
		Durchschn.	1.00	1.00	1.00	1.02	1.01	1.01	1.02
		Differenz		-0.01	0	0.02	-0.01	0.01	-0.01
2 Leberlappen-resektionen nach Milzex-stirpation	K-index	1	56.6	70.1	72.7	76.0	78.8	64.2	56.3
		2	49.5	78.1	76.3	89.4	84.1	74.0	70.7
		Durchschn.	53.2	73.8	74.2	82.6	81.4	69.7	63.4
		Differenz		25.6	24.5	32.4	26.5	27.4	22.4
	P-zahl	1	1.00	0.94	0.97	0.93	0.94	0.90	0.94
		2	1.00	0.97	0.90	0.86	0.85	0.87	0.87
		Durchschn.	1.00	0.96	0.94	0.90	0.90	0.89	0.91
		Differenz		-0.05	-0.06	-0.11	-0.11	-0.12	-0.10
2 Leberlappen resektionen	K-index	1	57.8	61.6	69.9	75.0	71.0	60.6	66.5
		2	48.7	77.1	77.7	80.2	66.7	60.6	57.3
		Durchschn.	52.8	68.5	73.8	77.8	68.3	60.6	61.7
		Differenz		8.7	12.5	18.5	13.2	5.1	6.5
	P-zahl	1	1.00	0.97	0.99	0.99	0.98	0.99	1.03
		2	1.00	0.81	0.93	0.93	0.90	0.90	0.93
		Durchschn.	1.00	0.89	0.96	0.95	0.94	0.95	0.98
		Differenz		-0.12	-0.04	-0.06	-0.07	-0.04	-0.02

TABELLE II

Einfluss der Milzexstirpation im langen Intervalle

		Nr. des Kaninchens	Vor Op.	Zeit nach Operation (Tage)							
				1	2	4	7	10	14	21	28
Kontrolle	K-index	1	49.5	51.8	45.0	44.0	49.8	39.8	51.0	53.8	
		2	45.6	47.3	46.3	40.0	39.3	35.3	46.9	55.5	58.6
		3	44.7	44.1	41.1	41.9	46.8	51.5	42.3	43.3	45.8
		Durchschn.	47.6	47.8	44.7	41.8	45.3	42.5	47.3	51.2	52.3
		Differenz		0.2	−2.9	−5.8	−2.3	-5.1	−0.3	3.6	4.7
	P-zahl	1	1.00	0.98	0.96	0.99	0.98	1.03	0.95	1.00	
		2	1.00	1.03	1.00	1.00	1.00	1.07	1.05	1.00	1.00
		3	1.00	1.04	0.99	1.04	0·98	1.05	1.00	1.00	1.00
		Durchschn.	1.00	1.02	0.98	1.01	0.99	1.05	1.00	1.00	1.00
		Differenz		0.02	-0.02	0.01	−0.01	0.05	0	0	0
Laparotomie	K-index	1	45.2	38.3	27.0	33.3	33.3	44.5	49.5	35.9	44.6
		2	43.5	32.5	27.2	34.1	31.0	34.1	43.7	64.2	48.8
		3	40.7	50.0	41.2	45.7	43.8	56.3	50.8	46.3	40.4
		Durchschn.	43.3	41.7	31.7	37.8	36.1	43.7	47.8	47.8	43.7
		Differenz		−1.8	−8.7	0.3	−4.9	5.5	4.8	8.1	−4.3
	P-zahl	1	1.00	1.02	1.05	1.02	1.06	1.03	1.03	1.03	0.98
		2	1.00	0.97	1.05	1.01	1.01	0.98	0.98	1.01	1.04
		3	1.00	1.01	1.01	0.98	1.00	0.99	1.01	1.02	1.00
		Durchschn.	1.00	1.00	1.04	1.00	1.02	1.00	1.01	1.02	1.01
		Differenz		−0.02	0.06	−0.01	0.03	−0.05	0.01	0.02	0.01
Milzexstirpation	K-index	1	53.2	68.8	56.6	53.3	46.5	41.7	46.7	45.8	42.8
		2	42.3	68.2	41.3	63.8	38.7	23.0	53.9	42.7	56.7
		Durchschn.	48.4	68.6	46.9	57.5	40.6	34.0	47.0	44.4	51.2
		Differenz		21.8	10.1	14.6	-0.6	−14.8	−5.9	−8.5	2.4
	P-zahl	1	1.00	1.06	0.98	0.98	0.95	1.00	1.06	0.98	1.01
		2	1.00	0.93	1.05	0.90	0.99	1.03	1.00	0.95	1.00
		Durchschn.	1.00	1.00	1.02	0.94	0.97	1.01	1.03	0.97	1.00
		Differenz		0	−0.02	−0.06	−0.05	0.01	0.02	−0.05	−0.01

Milzexstirpation reseziert, so sind eine Vermehrung des Kongorotindex und eine Verminderung der Prothrombinzahl auffälliger als bei 2 Leberlappenresektionen des gesunden Kaninchens.

Aus obigen Ergebnissen wird klar, dass die Funktion des RES gleich nach der Milzexstirpation herabgesetzt, dann kompensatorisch gesteigert wird und ungefähr nach 4 Wochen zum früheren Zustand zurückkehrt.

2. Einfluss der Milzpräparate

Aus Tabelle III sieht man, dass die Injektion des Extrakts einer ganzen Milz, der zentrifugierten Flüssigkeit der zergequetschten Kaninchenmilz mit 5 ccm physiologischer Kochsalzlösung, unter die Haut des gesunden Kaninchens, 3 Stunden nach der Injektion eine Verminderung des Kongorotindex verursacht, wobei die Prothrombinzahl fast unverändert bleibt, während eine Verminderung des Kongorotindex bei subkutaner Injektion eines Fünftels des obigen Milzextrakts nicht so deutlich ist.

TABELLE III

Einfluss der Milzpräparate

	Dose pro kg		Nr. des Kaninchens	Vor Inj.	Zeit nach Injektion (St.)		
					1	3	5
Milzextrakt	ganze Menge	K-index	1	52.0	68.0	42.6	43.3
			2	51.7	59.5	46.2	60.8
			Durchschn.	51.9	63.8	44.5	52.3
			Differenz		7.4	−18.4	−16.1
		P-zahl	1	1.00	1.03	1.00	0.97
			2	1.00	0.94	0.97	1.03
			Durchschn.	1.00	0.99	0.99	1.00
			Differenz		−0.01	−0.03	−0.04
	1/5 Menge	K-index	1	46.0	53.0	60.2	53.5
			2	45.1	50.6	52.2	54.0
			Durchschn.	45.7	51.9	56.6	53.7
			Differenz		1.7	−0.1	−8.4
		P-zahl	1	1.00	0.99	0.99	1.03
			2	1.00	1.01	0.98	1.02
			Durchschn.	1.00	1.00	0.99	1.03
			Differenz		0	-0.03	-0.01
Milzsin	1 ccm s.c.	K-index	1	66.8	55.4	69.4	80.0
			2	54.6	53.1	60.0	73.3
			Durchschn.	60.7	54.3	65.0	76.8
			Differenz		−18.1	−14.8	−1.4
		P-zahl	1	1.00	1.00	1.00	0.95
			2	1.00	0.99	0.98	0.96
			Durchschn.	1.00	1.00	0.99	0.96
			Differenz		0.01	0.02	−0.02
Retio A	1 EH s.c.	K-index	1	45.9	35.5	51.1	46.6
			2	42.7	41.6	43.7	50.0
			Durchschn.	44.3	38.2	47.4	47.9
			Differenz		−9.3	-1.1	-0.1
		P-zahl	1	1.00	0.99	1.02	0.98
			2	1.00	0.99	0.99	0.97
			Durchschn.	1.00	0.99	1.00	0.98
			Differenz		-0.02	-0.03	-0.05

Wenn man Milzsin (Nippon Shinyaku), das Milzhormon, in Menge von 1 ccm pro kg, oder Retio A (Takenaka Seiyaku), das Autohormon der reticuloendothelialen Zellen, in Menge von 1 Einheit unter die Haut des gesunden Kaninchens injiziert, so vermindert sich der Kongorotindex vorübergehend, die Prothrombinzahl bleibt aber fast unverändert.

Wie man aus Tabelle IV ersieht, sind eine Verminderung des Kongorotindex und eine Vermehrung der Prothrombinzahl bei subkutaner Injektion des Extrakts einer ganzen Milz 5 Stunden nach der Milzexstirpation sehr merklich, und sogar der Kongorotindex vermindert sich

TABELLE IV

Einfluss der Milzpräparate nach der Milzexstirpation

	Dose pro kg		Nr. des Kaninchens	Vor Milzexstirpation	5 St. nach Milzexstirpation	Zeit nach Injektion (St.) 1	3	5
Milzexstirpation		K-index	1	45.9	53.8	50.0	52.4	61.4
			2	42.1	46.5	55.8	52.3	55.0
			Durchschn.	44.0	49.5	53.0	52.5	57.7
			Differenz			3.5	2.8	8.2
		P-zahl	1	1.00	1.04	1.00	0.98	0.99
			2	1.00	1.02	0.98	0.98	0.99
			Durchschn.	1.00	1.03	0.99	0.98	0.99
			Differenz		(1.00)	-0.01	-0.02	-0.01
Milzextrakt nach Milzexstirpation	ganze Menge	K-index	1	72.1	85.1	59.5	73.3	60.8
			2	57.7	68.6	65.0	70.6	73.0
			Durchschn.	63.1	73.3	62.8	71.7	68.3
			Differenz			-14.0	-4.4	-13.2
		P-zahl	1	1.00	1.02	1.18	1.21	1.22
			2	1.00	1.01	1.01	1.00	1.03
			Durchschn.	1.00	1.01	1.09	1.10	1.12
			Differenz		(1.00)	0.10	0.12	0.13
Milzsin nach Milzexstirpation	1 ccm s.c.	K-index	1	52.2	59.8	60.8	59.1	65.0
			2	51.4	65.8	71.2	66.7	75.0
			3	41.1	73.2	74.8	66.7	66.7
			Durchschn.	48.2	67.2	69.8	64.4	68.8
			Differenz			-0.9	-5.6	-6.6
		P-zahl	1	1.00	0.98	1.01	1.02	1.08
			2	1.00	0.98	1.03	1.07	1.06
			3	1.00	1.01	0.97	1.01	1.06
			Durchschn.	1.00	0.99	1.00	1.03	1.07
			Differenz		(1.00)	0.01	0.05	0.08
Glukose nach Milzexstirpation	25% 10 ccm i.v.	K-index	1	63.8	88.8	64.3	80.3	83.3
			2	56.6	89.6	73.3	78.4	65.2
			Durchschn.	60.3	89.3	67.3	79.6	73.1
			Differenz			-25.5	-12.5	-24.4
		P-zahl	1	1.00	0.99	0.98	1.00	1.04
			2	1.00	0.99	0.97	1.00	0.98
			Durchschn.	1.00	0.99	0.98	1.00	1.01
			Differenz		(1.00)	-0.01	0.02	0.02
Hypon nacn Milzexstirpation	50% 1 ccm i.v.	K-index	1	65.2	88.8	56.8	72.8	75.0
			2	51.2	71.7	69.5	78.8	82.3
			Durchschn.	56.9	79.4	63.2	75.8	77.3
			Differenz			-19.7	-6.4	-10.3
		P-zahl	1	1.00	1.03	0.97	0.99	0.95
			2	1.00	0.98	1.07	1.01	1.01
			Durchschn.	1.00	1.00	1.02	1.00	0.98
			Differenz			0.03	0.02	-0.01

leichtgradig, die Prothrombinzahl aber vermehrt sich nach Milzsininjektion beim entmilzten Kaninchen. Aber die Injektion von 25% Glukose in Menge von 10 ccm pro kg oder von 50% Hypon (Natriumthiosulfat) in Menge von 1 ccm pro kg in die Vene des entmilzten Kaninchens verursacht eine Verminderung des Kongorotindex, während die Prothrombinzahl fast unverändert bleibt.

Aus diesen Versuchsergebnissen ist zu schliessen, dass diese Milzpräparate einen günstigen Einfluss auf die Funktion des RES sowohl beim gesunden als auch beim entmilzten Kaninchen ausüben.

Diskussion

Nach Aschoff[1] gehören die Reticulmzellen der Milzpulpa und die Sinusendothelien der Milz zum RES im engeren Sinne und die Splenozyten zum RES im weiteren Sinne; deshalb wird allgemein anerkannt, dass die Milz ein wichtiges Organ des RES ist. Mehrere Autoren nehmen an, dass die Milzexstirpation die Funktion des RES herabsetzt (Adler,[5] Leites,[6] Yuki[7], Tokuno,[8] Kinoshita,[9] Goebel und Miller[10]). Aber Noma[11] und Tomoda[12] betonen, dass diese Herabsetzung der Funktion des RES nach der Milzexstirpation durch sonstige reticuloendotheliale Zellen wie Kupffersche Sternzellen in der Leber kompensiert werden kann. Weiter weisen Goebel[10] und Rindone[13] histologisch nach, dass die Sternzellen nach der Milzexstirpation hypertrophieren und wuchern.

Aus unseren Versuchsergebnissen ist es klar geworden, dass die Funktion des RES sofort nach der Milzexstirpation herabgesetzt, dann kompensatorisch gesteigert wird und ungefähr nach 4 Wochen zum früheren Zustand zurückkehrt. Dass die Leberzellenfunktion mehr oder weniger zu einer Verminderung neigt, nicht nur nach der Milzexstirpation, sondern auch nach 2 Leberlappenresektionen des entmilzten Kaninchens, lässt vermuten, dass die Milz eine kompensatorische Funktion für die Leber hat.

Schon in der II. Mitteilung[3] dieser Arbeit fanden wir, dass die intravenöse Injektion von Glukose oder Natriumthiosulfat die Funktion des RES steigert. Aber Tokuno[8] bemerkte, dass das Milzextrakt die Funktion des RES erhöht, und weiter betonte Torrioli,[14] dass die reticuloendothelialen Zellen durch die intravenöse Injektion des Milzextrakts wuchern.

Nach unseren diesbezüglichen Ergebnissen wird festgestellt, dass das Milzextrakt, Milzsin und Retio A zwar nur die Funktion des RES des gesunden Kaninchens steigern kann, aber das Milzextrakt und Milzsin sowohl die Funktion des RES als auch die Leberzellenfunktion des entmilzten Kaninchens erhöhen, während Glukose und Hypon nur die Funk-

tion des RES des gesunden sowie des entmilzten Kaninchens fördern kann.

Aus obenerwähnten Versuchsergebnissen ist zu vermuten, dass die Milz an der Funktion des RES beteiligt ist, und zwar nicht nur als Teilorgan des RES, sondern auch als inkretorisches Organ des stimulierenden Hormons des RES.

Zusammenfassung

Fassen wir obige Versuchsergebnisse zusammen, so kommen wir zu folgendem Schluss:

1. Nach der Milzexstirpation vermehrt sich der Kongorotindex bis etwa zum 7. Tage, dann vermindert er sich und kehrt ungefähr nach 4 Wochen zum früheren Zustand wieder zurück, während eine Verminderung der Prothrombinzahl nicht so auffallend ist.

2. Nach 2 Leberlappenresektionen des entmilzten Kaninchens sind eine Verminderung des Kongorotindex und eine Verminderung der Prothrombinzahl auffälliger als diejenigen des gesunden Kaninchens.

3. Bei Injektion von Milzextrakt, Milzsin und Retio A vermindert sich der Kongorotindex des gesunden Kaninchens, während die Prothrombinzahl fast unverändert bleibt. Aber eine Verminderung des Kongorotindex und eine Vermehrung der Prothrombinzahl des entmilzten Kaninchens sind sehr merklich bei Injektion von Milzextrakt und Milzsin. Weiter bei Injektion von Glukose und Hypon in die Vene des entmilzten Kaninchens vermindert sich der Kongorotindex, die Prothrombinzahl bleibt aber fast unverändert.

4. Daraus ist zu vermuten, dass die Funktion des RES sich sofort nach der Milzexstirpation herabsetzt, dann kompensatorisch sich steigert, um schliesslich den früheren Zusand wiederherzustellen, und dass die Milz nicht nur einen stimulierenden Faktor für die Funktion des RES enthält, sondern auch ein stimulierendes Hormon sezerniert.

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V. Mitteilung

Einfluss verschiedener Hormone und Vitamine auf die Funktion des RES und der Leber

Von

Shoichi Yamagata, Ichiro Arai, Kihachi Unoura, Toshio Aratani,
(山形 敞一) (荒井 一郎) (鵜浦 喜八) (荒谷 利夫)
Kiyomi Miura und **Kisao Otomo**
(三浦 清美) (大友 喜佐雄)

Aus der Med. Klinik von Prof. Dr. T. Kurokawa, Tohoku Universität, Sendai

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Es liegt sehr nahe, zu vermuten, dass Hormone und Vitamine einen gewissen Einfluss auf die Funktion des RES haben, weil beide eine regulatorische Wirkung auf den Stoffwechsel des Lebewesens ausüben. Heutzutage wird eine engere Beziehung zwischen dem vegetativen Nervensystem und den inkretorischen Organen angenommen. In der III. Mitteilung[1] dieser Untersuchung ist klargestellt geworden, dass der Sympathicus auf die Funktion der Leberzellen fördernd und auf diejenige des RES einschliesslich der Sternzellen hemmend, dagegen der Parasympathicus auf die erstere hemmend und auf die letztere fördernd einwirkt. Aber die Mitteilungen über den Einfluss verschiedener Hormone und Vitamine auf die Funktion des RES und der Leber sind je nach den Autoren ganz verschieden.

Infolgedessen haben wir in der vorliegenden Untersuchung versucht, den Wirkungsmechanismus der Hormone und Vitamine zu klären. Die Auswahl der Versuchstiere sowie die Methodik war die gleiche wie bei der früheren Mitteilung.[2]

Versuchsergebnisse

1. Einfluss verschiedener Hormone

Wie man aus Tabelle I, II und III ersieht, vermindert sich durch die Injektion von Hypophorin (Hypophysenvorderlappenhormon) in Menge von 1 ccm pro kg, Pituitrin (Hypophysenhinterlappenhormon) in Menge von 0.3 ccm pro kg, Syncorta (DOCA) in Menge von 2.5 mg pro kg oder Ovoglandol (Ovarialextrakt) in Menge von 0.5 ccm pro kg der Kongorotindex und die Prothrombinzahl bleibt unverändert, dagegen

TABELLE I

Einfluss der Hypophysen-und Nebennierenrindenhormone

	Dose (pro kg)		Nr. des Kaninchens	Vor Inj.	Zeit nach Injektion (St.) 1	3	5
Hypophorin	1 ccm s.c.	K-index	1	59.2	56.7	66.7	63.6
			2	53.8	46.3	48.7	54.0
			Durchschn.	56.7	51.7	59.0	59.7
			Differenz		-16.8	-16.8	-14.3
		P-zahl	1	1.00	1.04	1.03	1.02
			2	1.00	1.04	1.04	1.00
			Durchschn.	1.00	1.02	1·04	1.01
			Differenz		0.01	0.02	-0.02
Acthar	1 mg i.m.	K-index	1	55.5	43.5	57.2	69.7
			2	41.9	48.8	74.0	60.1
			Durchschn.	48.6	46.1	67.3	63.5
			Differenz		-5.7	14.4	11.2
		P-zahl	1	1.00	1.07	1.05	1.00
			2	1.00	1.05	1.01	1.01
			Durchschn.	1.00	1.06	1.03	1.00
			Differenz		0.05	0	-0.03
	2 mg i.m.	K-index	1	64.5	87.5	75.5	77.5
			2	41.9	37.0	60.8	68.8
			Durchschn.	54.0	61.6	68.8	74.2
			Differenz		4.4	10.1	16.5
		P-zahl	1	1.00	1.12	1.07	1.05
			2	1.00	1.03	1.06	1.10
			Durchschn.	1.00	1.07	1.06	1.07
			Differenz		0.06	0.03	0.04
Syncorta	2.5 mg i.m.	K-index	1	65.3	73.7	50.0	55.2
			2	64.3	55.0	71.2	60.0
			Durchschn.	64.8	64.3	63.0	58.3
			Differenz		-12.3	-20.9	-24.0
		P-zahl	1	1.00	0.99	1.03	1.02
			2	1.00	0.99	1.00	1.01
			Durchschn.	1.00	0.99	1.02	1.02
			Differenz		-0.02	0	-0.01

verursacht die Injektion von Acthar (Adrenocorticotropes Hormon) in Menge von 1 oder 2 mg pro kg eine Verminderung des Kongorotindex sowie der Prothrombinzahl. Weiterhin ruft die Injektion von Rindex (Nebennierenrindenhormon) in Menge von 1 ccm pro kg oder Thyroxin in Menge von 0.5 ccm pro kg eine Verminderung des Kongorotindex sowie der Prothrombinzahl hervor.

Wenn man Praehormon (Gonadotropes Hypophysenvorderlappenhormon) in Menge von 100 Einheiten (EH) pro kg, Synahorin (Gonadotropes Placentarhormon) in Menge von 10 EH pro kg, Rohormon (Follikelhormon) in Menge von 0.5 mg pro kg oder Progenin (Corpus-

TABELLE II

Einfluss der Sexualhormone

	Dose (pro kg)		Nr. des Kaninchens	Vor Inj.	Zeit nach Injektion (St.) 1	3	5
Praehormon	100 EH s.c.	K-index	1	62.6	79.6	54.6	54.6
			2	50.1	79.6	62.3	54.3
			Durchschn.	56.6	79.6	58.1	54.6
			Differenz		11.2	-17.6	-19.5
		P-zahl	1	1.00	0.95	0.99	1.07
			2	1.00	0.94	1.03	1.05
			Durchschn.	1.00	0.95	1.01	1.06
			Differenz		-0.04	0.04	0.08
Synahorin	10 EH i.m.	K-index	1	56.3	60.4	62.6	69.6
			2	55.6	70.9	67.7	78.1
			Durchschn.	56.2	66.2	65.8	74.7
			Differenz		-1.8	-9.9	1.0
		P-zahl	1	1.00	1.08	1.08	1.08
			2	1.00	1.03	0.96	0.95
			Durchschn.	1.00	1.06	1.02	1.02
			Differenz		0.07	0.05	0.04
Rohormon	0.5 mg i.m.	K-index	1	72.1	51.2	72.8	72.4
			2	57.7	60.9	62.2	68.1
			Durchschn.	65.2	54.8	68.7	78.1
			Differenz		-22.2	-15.6	-4.6
		P-zahl	1	1.00	0.98	1.02	1.09
			2	1.00	0.98	1.00	1.06
			Durchschn.	1.00	0.98	1.01	1.08
			Differenz		-0.01	0.04	0.10
Progenin	1 mg i.m.	K-index	1	56.8	70.4	71.5	48.5
			2	49.0	58.6	56.7	58.6
			Durchschn.	52.8	65.1	63.7	52.3
			Differenz		0.5	-8.2	-17.0
		P-zahl	1	1.00	1.01	1.06	1.12
			2	1.00	1.02	0.96	0.96
			Durchschn.	1.00	1.02	1.01	1.04
			Differenz		0.03	0.04	0.06
Amolysin	1 mg i.m.	K-index	1	45.5	70.4	59.4	73.9
			2	42.9	76.3	68.7	70.9
			Durchschn.	44.7	73.0	64.2	72.7
			Differenz		16.5	0.4	10.5
		P-zahl	1	1.00	1.12	1.10	1.13
			2	1.00	0.99	1.03	1.01
			Durchschn.	1.00	1.01	1.07	1.07
			Differenz		0.07	0.10	0.09
	0.5 mg i.m.	K-index	1	58.6	54.1	60.0	80.1
			2	56.0	49.5	54.1	64.5
			Durchschn.	56.3	51.6	57.1	71.1
			Differenz		-16.5	-18.3	-2.7
		P-zahl	1	1.00	1.04	1.08	1.07
			2	1.00	0.92	0.96	1.01
			Durchschn.	1.00	0.98	1.04	1.04
			Differenz		-0.01	0.05	0.06

TABELLE III

Einfluss verschiedener Hormone

	Dose (pro kg)		Nr. des Kaninchens	Vor Inj.	Zeit nach Injektion (St.)					
					3	5	8	12	24	48
Pituitrin	0.3 ccm s.c.	K-index	1	50.6	41.0	50.0	55.0	39.6	57.2	71.6
			2	31.2	40.2	47.9	43.2	39.9	41.7	55.0
			Durchschn.	40.9	40.7	49.0	49.2	39.7	49.2	63.7
			Differenz		-7.0	-5.4	-2.5	-7.7	1.5	14.4
		P-zahl	1	1.00	0.97	0.95	0.98	0.96	1.00	0.97
			2	1.00	0.97	0.97	0.98	0.98	0.99	0.97
			Durchschn.	1.00	0.97	0.96	0.98	0.97	1.00	0.97
			Differenz		-0.03	-0.03	-0.03	-0.03	0	-0.03
Rindex	1 ccm s.c.	K-index	1	45.3	48.0	55.0	53.5	47.6	43.6	55.2
			2	45.0	46.3	58.4	54.8	49.2	54.1	55.1
			Durchschn.	45.1	47.2	57.3	54.1	43.0	48.7	55.2
			Differenz		-4.7	-1.3	-1.8	-9.6	-3.2	1.3
		P-zahl	1	1.00	1.02	0.98	1.04	0.92	0.96	0.99
			2	1.00	1.00	0.97	1.00	0.86	0.96	0.95
			Durchschn.	1.00	1.01	0.98	1.02	0.89	0.96	0.97
			Differenz		0.01	-0.01	0.01	-0.11	-0.04	-0.03
Thyroxin	0.5 ccm s.c.	K-index	1	52.2	52.3	48.8	40.5	40.5	35.9	26.9
			2	37.6	48.8	43.8	45.0	46.0	40.3	20.3
			Durchschn.	45.3	50.5	46.3	42.8	45.7	38.0	23.9
			Differenz		-3.9	-3.5	-6.6	-5.0	-9.7	-23.2
		P-zahl	1	1.00	0.82	0.88	0.89	1.01	1.02	1.02
			2	1.00	0.90	0.89	0.89	0.98	1.00	1.01
			Durchschn.	1.00	0.86	0.89	0.89	1.00	1.01	1.02
			Differenz		-0.13	-0.11	-0.14	-0.04	-0.02	0.03
Ovo-glandol	0.5 ccm s.c.	K-index	1	52.5	52.0	62.0	59.9	53.8	48.6	46.8
			2	51.5	52.3	63.6	62.0	57.5	61.8	50.0
			Durchschn.	51.9	52.2	62.8	60.9	55.8	55.1	48.5
			Differenz		-6.5	-2.6	-1.8	-3.7	-3.6	-12.2
		P-zahl	1	1.00	1.00	1.03	0.98	1.01	1.02	0.96
			2	1.00	1.02	0.98	1.02	0.99	0.97	0.91
			Durchschn.	1.00	1.01	1.01	1.00	1.00	1.00	0.94
			Differenz		0.01	0.02	-0.01	0	0	-0.03
Perandren	2.5 mg s.c.	K-index	1	40.6	46.9	62.0	68.5	46.0	60.0	64.5
			2	32.7	38.1	36.6	37.5	40.9	42.5	37.6
			Durchschn.	36.7	42.5	49.3	53.0	43.5	51.3	51.1
			Differenz		-2.3	3.5	8.8	0.3	10.1	11.1
		P-zahl	1	1.00	1.02	0.97	0.93	0.94	1.05	1.00
			2	1.00	1.00	0.93	0.93	0.93	0.99	1.00
			Durchschn.	1.00	1.01	0.95	0.93	0.94	1.02	1.00
			Differenz		0.02	-0.05	-0.09	-0.07	0.01	0

luteumhormon) in Menge von 1 mg pro kg bei weiblichen Kaninchen sowie Amolysin (Testikelhormon) in Menge von 0.5 mg pro kg bei männlichen injiziert, so vermindert sich der Kongorotindex und die Prothrom-

binzahl vermehrt sich. In ähnlicher Weise wie bei Acthar verursacht die Injektion von Amolysin in Menge von 1 mg pro kg eine Vermehrung des Kongorotindex sowie der Prothrombinzahl, aber die Injektion von Perandren (Testikelhormon) in Menge von 2.5 mg pro kg ruft bei männlichen Kaninchen eine Vermehrung des Kongorotindex und eine Verminderung der Prothrombinzahl hervor.

Betreffs der Pankreashormone ist die Reaktion nicht einheitlich, wie aus Tabelle IV zu sehen ist: die Insulininjektion in Menge von 0.5 oder 2 EH pro kg setzt den Kongorotindex herab, dagegen bleibt der Kon-

TABELLE IV

Einfluss der Pankreashormone

	Dose (pro kg)		Nr. des Kaninchens	Vor Inj.	Zeit nach Injektion (St.)		
					1	3	5
Insulin	0.25 EH s.c.	K-index	1	56.2	56.8	62.8	65.2
			2	48.7	53.0	60.0	46.6
			Durchschn.	51.5	55.5	61.3	55.8
			Differenz		0.3	5.5	0.6
		P-zahl	1	1.00	1.08	1.03	1.02
			2	1.00	0.97	1.02	1.03
			Durchschn.	1.00	1.03	1.03	1.03
			Differenz		0.02	0.01	-0.01
	2 EH s.c.	K-index	1	43.9	51.5	53.2	59.3
			2	40.8	42.8	47.7	45.4
			Durchschn.	42.3	46.9	50.6	52.0
			Differenz		-9.2	-5.4	0.5
		P-zahl	1	1.00	1.01	0.98	1.01
			2	1.00	1.01	0.97	1.01
			Durchschn.	1.00	1.01	0.98	1.01
			Differenz		0.01	-0.01	-0.01
Padutin	1 EH i.m.	K-index	1	52.6	64.8	62.5	63.6
			2	38.4	48.3	57.2	53.5
			Durchschn.	45.9	57.5	59.8	58.8
			Differenz		7.1	2.8	-2.6
		P-zahl	1	1.00	1.01	1.10	1.14
			2	1.00	1.01	1.10	1.01
			Durchschn.	1.00	1.01	1.10	1.08
			Differenz		0	0.08	0.04
	2 EH i.m.	K-index	1	66.8	75.0	56.2	68.2
			2	61.8	68.8	69.7	70.0
			Durchschn.	64.4	72.2	62.3	71.4
			Differenz		3.3	-13.1	-9.5
		P-zahl	1	1.00	1.11	1.02	1.03
			2	1.00	0.94	0.96	1.03
			Durchschn.	1.00	1.03	0.99	1.53
			Differenz		0.02	-0.03	-0.01

gorotindex unbeeinflusst durch Insulin in Menge von 0.25 EH pro kg wobei auch die Prothrombinzahl ganz unverändert bleibt. Weiter ähnelt die Wirkung des Padutins (Kreislaufhormon des Pankreas) in Menge von 1 EH pro. kg derjenigen von Acthar, dagegen wirkt eine Dosierung von 2 EH pro kg in ähnlicher Weise wie bei Syncorta.

Daraus wird klar, dass diese Hormone, nicht aber Acthar, Amolysin in grosser Menge und Perandren, die Funktion des RES steigern, dagegen wird die Leberzellenfunktion durch Acthar, Praehormon, Rohormon, Progenin und Amolysin gereizt.

2. *Einfluss verschiedener Vitamine*

Aus Tabelle V sieht man, dass die Injektion von Vitadol in Menge von 0.5 ccm pro kg (Vitamin A 15000 EH mit Vitamin D 3000 EH) eine deutliche Verminderung des Kongorotindex und eine Vermehrung der Prothrombinzahl veranlasst. In gleicher Weise vermindert sich der Kongorotindex und sie Prothrombinzahl vermehrt sich, wie Tabelle V, VI und VII zeigt, bei Injektion von Metabolin (Vitamin B_1) in Menge von

TABELLE V

Einfluss des Vitamin A und Seiner verwandten Vitamine

	Dose (pro kg.)		Nr. des Kaninchens	Vor Inj.	Zeit nach Injektion (St.)		
					1	3	5
Vitadol	0.5 ccm i.m.	K-index	1	70.0	69.4	72.3	76.4
			2	66.5	56.6	68.4	73.7
			Durchschn.	68.2	63.2	70.5	75.3
			Differenz		-16.8	-16.8	-10.4
		P-zahl	1	1.00	1.09	1.12	1.02
			2	1.00	0.98	1.01	1.00
			Durchschn.	1.00	1.04	1.07	1.01
			Differenz		0.05	0.10	0.03
Ovoral	5000 EH i.m.	K-index	1	52.8	48.6	56.2	73.5
			2	29.0	37.5	42.8	47.3
			Durchschn.	42.6	44.0	51.0	64.0
			Differenz		-10.4	-10.7	3.9
		P-zahl	1	1.00	1.06	1.00	0.98
			2	1.00	1.00	1.00	0.99
			Durchschn.	1.00	1.03	1.00	0.99
			Differenz		0.04	0.03	0.01
Juvela	50 mg i.m.	K-index	1	55.7	51.1	50.0	59.2
			2	53.3	57.2	72.0	77.8
			Durchschn.	53.8	54.2	61.7	70.6
			Differenz		-4.9	-3.1	0.3
		P-zahl	1	1.00	0.98	1.05	0.97
			2	1.00	0.99	1.00	0.96
			Durchschn.	1.00	0.99	1.03	0.97
			Differenz		-0.02	0	-0.06

Tabelle VI
Einfluss der Vitamin B-Komplexe

	Dose (pro kg)		Nr. der Kaninchens	Vor Inj.	Zeit nach Injektion (St.) 1	3	5
Metabolin	5 mg i.v.	K-index	1	36.4	51.6	45.6	52.3
			2	29.7	38.7	34.2	33.2
			Durchschn.	33.4	44.9	39.7	42.8
			Differenz		-0.3	-12.8	-8.1
		P-zahl	1	1.00	1.08	1.04	1.08
			2	1.00	1.06	1.06	1.00
			Durchschn.	1.00	1.07	1.05	1.04
			Differenz		0.08	0.08	0.06
Bisulase	5 mg i.v.	K-index	1	50.0	51.4	54.9	55.5
			2	44.3	55.5	55.7	48.2
			Durchschn.	45.8	53.3	55.8	51.6
			Differenz		-4.3	-9.1	-11.7
		P-zahl	1	1.00	0.99	1.03	1.09
			2	1.00	0.99	1.04	1.04
			Durchschn.	1.00	0.99	1.04	1.07
			Differenz		0	0.07	0.09
Aderoxin	10 mg s.c.	K-index	1	56.3	64.5	71.2	68.3
			2	46.9	58.8	66.6	61.5
			Durchschn.	51.5	61.6	67.3	65.4
			Differenz		-1.7	-3.3	-3.6
		P-zahl	1	1.00	1.07	1.02	1.01
			2	1.00	1.00	1.00	1.01
			Durchschn.	1.00	1.04	1.01	1.01
			Differenz		0.05	0.04	0.03
Fresmin	10 i.m.	K-index	1	67.6	68.2	57.2	81.1
			2	55.5	62.3	68.2	78.5
			Durchschn.	61.0	65.2	62.5	79.8
			Differenz		-7.6	-17.6	0.9
		P-zahl	1	1.00	0.98	1.00	1.08
			1	1.00	0.98	0.98	0.99
			Durchschn.	1.00	0.98	0.99	1.04
			Differenz		-0.01	0.02	0.06
Niamide	50 mg s.c.	K-index	1	57.3	49.3	64.4	71.4
			2	49.5	40.8	56.8	53.8
			Durchschn.	53.2	44.9	60.3	62.2
			Differenz		-20.1	-12.0	-8.5
		K-index	1	1.00	0.84	0.86	1.01
			2	1.00	0.83	0.81	0.88
			Durchschn.	1.00	0.84	0.84	0.95
			Differenz		-0.15	-0.13	-0.03
Folical	15 mg s.c.	K-index	1	55.8	61.7	63.2	68.7
			2	53.5	55.5	65.3	72.8
			Durchschn.	54.4	58.7	64.2	70.8
			Differenz		-7.7	-9.4	-1.3
		P-zahl	1	1.00	1.06	1.09	1.05
			2	1.00	1.07	1.08	1.03
			Durchschn.	1.00	1.07	1.09	1.04
			Differenz		0.08	0.12	0.06

TABELLE VII

Einfluss des Vitamin C und Seiner verwandten Vitamine

	Dose (pro kg)]		Nr. des Kaninchens	Vor Inj.	Zeit nach Injektion (St.)		
					1	3	5
Redoxon	50 mg i.v.	K-index	1	63.3	68.1	72.3	70.5
			2	53.1	55.0	56.7	68.0
			Durchschn.	59.1	62.2	65.4	69.5
			Differenz		-8.7	-12.8	-7.1
		P-zahl	1	1.00	1.04	1.01	1.03
			2	1.00	1.00	1.01	1.04
			Durchschn.	1.00	1.02	1.01	1.04
			Differenz		0.03	0.04	0.06
Kativ	10 mg i.v.	K-index	1	63.3	70.5	69.7	76.8
			2	51.3	51.6	44.5	65.0
			Durchschn.	56.8	60.7	55.2	72.1
			Differenz		-7.9	-11.4	-2.2
		P-zahl	1	1.00	1.02	1.12	1.17
			2	1.00	1.09	1.09	1.06
			Durchschn.	1.00	1.06	1.11	1.12
			Differenz		0.07	0.14	0.14
Hesperin	50 mg i.v.	K-index	1	50.5	55.0	60.0	57.7
			2	41.8	56.5	64.8	57.7
			Durchschn.	46.5	55.8	62.2	57.7
			Differenz		-2.5	-3.4	-6.3
		P-zahl	1	1.00	1.08	1.03	1.03
			2	1.00	1.00	0.99	0.96
			Durchschn.	1.00	1.04	1.01	1.00
			Differenz		0.05	0.04	0.02

5 mg pro kg, Bisulase (Vitamin B_2) in Menge von 5 mg pro kg, Fresmin (Vitamin B_{12}) in Menge von 10 mg pro kg, Folical (Blättersäure) in Menge von 15 mg pro kg, Redoxon (Vitamin C) in Menge von 50 mg pro kg oder Kativ (Vitamin K) in Menge von 10 mg pro kg, beides bis zu einem gewissen Grade. Weiterhin vermindert sich der Kongorotindex nach der Injektion von Aderoxin (Vitamin B_6) in Menge von 10 mg pro kg, Niamide (Nikotinsaures Amid) in Menge von 50 mg pro kg, Ovoral (Vitamin D) in Menge von 5000 EH pro kg oder Hesperin (Vitamin P) in Menge von 50 mg pro kg, während die Prothrombinzahl fast unverändert bleibt. Im Gegensatz dazu verursacht die Injektion von Juvela (Vitamin E) in Menge von 50 mg pro kg eine leichte Verminderung des Kongorotinedex sowie der Prothrombinzahl.

Aus diesen Versuchsergebnissen lässt sich schliessen, dass diese Vitamine die Funktion des RES steigern, jedes bis zu einem gewissen Grade, dagegen die Leberzellenfunktion durch Vitadol, Metabolin, Bisulase, Fresmin, Folical, Redoxon und Kativ angeregt wird.

TABELLE VIII

Einfluss des Luminals auf die Wirkung des Hormons und Vitamins

	Dose (pro kg)		Nr. des Kaninchens	Vor Inj.	Zeit nach Injektion (St.)		
					1	3	5
Luminal	30 mg i.m.	K-index	1	50.0	55.6	68.0	65.2
			2	45.8	70.6	66.7	64.8
			Durchschn.	47.8	63.0	67.3	65.1
			Differenz		13.0	15.2	13.6
		P-zahl	1	1.00	1.00	1.00	0.96
			2	1.00	1.00	1.00	0.96
			Durchschn.	1.00	1.00	1.00	0.94
			Differenz		0	0.01	-0.07
Hypophorin	1 ccm s.c.	K-index	1	59.2	56.7	66.7	63.6
			2	53.8	46.3	48.7	54.0
			Durchschn.	56.7	51.7	59.0	59.7
			Differenz		-16.8	-16.8	-14.3
		P-zahl	1	1.00	1.04	1.03	1.02
			2	1.00	1.00	1.04	1.00
			Durchschn.	1.00	1.02	1.03	1.01
			Differenz		0.01	0	-0.02
Hypophorin nach Luminal	Hypophorin 1 ccm s.c. Luminal 30 mg i.m.	K-index	1	60.0	53.3	61.0	75.0
			2	52.1	55.2	60.0	70.3
			Durchschn.	56.3	54.2	60.7	72.3
			Differenz		-13.9	-14.8	-1.5
		P-zahl	1	1.00	1.00	1.06	1.09
			2	1.00	0.96	0.90	1.00
			Durchschn.	1.00	0.98	0.98	1.05
			Differenz		-0.03	-0.05	0.02
Vitadol	0.5 ccm i.m.	K-index	1	70.0	69.4	72.3	76.4
			2	66.5	56.6	68.4	73.7
			Durchschn.	68.2	63.2	70.5	75.3
			Differenz		-16.8	-16.8	-10.4
		P-zahl	1	1.00	1.09	1.12	1.02
			2	1.00	0.98	1.01	1.00
			Durchschn.	1.00	1.04	1.07	1.01
			Differenz		0.05	0.10	0.03
Vitadol nach Luminal	Vitadol 0.5 ccm i.m. Luminal 30 mg i.m.	K-index	1	54.8	76.2	59.7	62.5
			2	51.8	57.2	76.0	75.1
			Durchschn.	53.2	65.8	67.0	68.2
			Differenz		0.8	-5.3	-2.5
		P-zahl	1	1.00	1.04	1.00	1.02
			2	1.00	0.99	0.92	0.96
			Durchschn.	1.00	1.02	0.96	0.99
			Differenz		0.01	-0.09	-0.06

3. Einfluss des Luminals auf die Wirkung des Hormons sowie des Vitamins

Schon in der III. Mitteilung[1] dieser Untersuchung berichteten wir,

dass die Luminalinjektion in Menge von 30 mg pro kg eine Vermehrung des Kongorotindex sowie eine Verminderung der Prothrombinzahl hervorruft. Aber man ersieht aus Tabelle VIII, dass die obenerwähnte Verminderung des Kongorotindex bei Hypophorin durch Luminal nicht gehemmt wird, dagegen die obige Wirkung bei Vitadol durch Luminal ganz aufgehoben wird. In beiden Fällen vermindert sich die Prothrombinzahl sehr merklich durch Luminal.

Daraus ist ersichtlich, dass Hypophorin die Funktion des RES direkt steigert, im Gegensatz dazu aber Vitadol mittelst des vegetativen Nervenzentrums die Funktion des RES stimuliert.

Diskussion

Schon in der III. Mitteilung[1] dieser Arbeit berichteten wir über eine engere Beziehung zwischen dem vegetativen Nervensystem und der Funktion des RES und der Leber. Auch Horster[3] und Okinaka[4] wiesen auf das Vorhandensein eines innigen Zusammenhanges des vegetativen Nervensystems mit den inkretorischen Organen hin. Aber die Meinungen der Autoren gehen auseinander betreffs der Beziehung zwischen den inkretorischen Organen und der Funktion des RES. Bei Blockade des RES wies Takeda[5] eine Steigerung des Hypophysenvorderlappens histologisch nach und darüber hinaus beobachteten Akanuma und To[6] eine dabei auftretende Steigerung der Schilddrüsen. Vor allem betonten Goldzieher und Hirschhorn,[7] dass die Resultate über obige Beziehung sehr abweichend sind je nach den verschiedenen Hormonpräparaten oder Funktionsprüfungsmethoden. In Bezug auf die Hypophyse ist das obenerwähnte Verhältnis aber besonders kompliziert. Takahashi[8] und Narimatsu[9] bemerkten eine Funktionssteigerung des RES nach der Hypophysenexstirpation, und zugleich Tangl[10], Takahashi[8] und Narimatsu[9] eine Erniedrigung durch Injektion des Hypophysenextrakts. Betreffs der Vorderlappenhormone berichteten Haendel und Malet[11] über eine Steigerung der Funktion des RES und Kinoshita[12] bemerkte eine Herabsetzung, während Takahashi[8] und Tsunoda[13] dabei die Unveränderlichkeit betonten. Weiterhin beobachtete Narimatsu[9] eine Herabsetzung der Funktion des RES durch Wachstumshormon und eine Steigerung durch gonadotropes Hormon. Bei Darreichung der Hinterlappenhormone bemerkten Leites[14] und Kinoshita[12] eine Funktionssteigerung des RES, dagegen Saxl[15] und Takahashi[8] eine Herabsetzung der Funktion, während Goldzieher und Hirschhorn[7] deren Unveränderlichkeit betonten. Nach Kinoshita[12] und Fukuoka[16] verursacht das Nebennierenrindenhormon eine Funktionserhöhung des RES, aber Tsurui[17] behauptete, dass diese Funktion durch grosse Dosierung sich erniedrigt, jedoch durch nachfolgende Injektion in kleiner Menge gesteigert wird. Nach der Schilddrüsen-

exstirpation fanden manche Autoren eine Herabsetztung der Funktion des RES (Jaffe[18], Kinoshita[12], Miyamura[19], Kamada[20], Uchino[21]), umgekehrt berichteten Leites und Riabow[14] über deren Steigerung. In ähnlicher Weise beobachteten einige eine Funktionssteigerung des RES durch Schilddrüsenhormon (Saxl[15], Miyamura[19], Kamada[20], Haendel[11], Kinoshita[12]), andere Autoren dagegen eine Herabsetzung (Tangl[10], Goldzieher[7], Uchino[21]). Nach der Ovarialexstirpation beobachteten Leites[14] und Tsunoda[13] eine Funktionssteigerung des RES, Kinoshita[12] und Uchino[21] umgekehrt eine Herabsetzung. Weiter fand Kinoshita[12] eine Herabsetzung der Funktion des RES nach der Hodenexstirpation, aber Leites[14] und Kamada[20] keine Beeinflussung. Die Meinungen der Autoren über die Wirkung der Geschlechtshormone sind in ähnlicher Weise sehr mannigfaltig. Kamada[20], Fukuoka und Mori[16] beobachteten eine Funktionssteigerung des RES durch männliches Hormon, dagegen Tangl[10] eine Herabsetzung. Bei den weiblichen Hormonen bemerkten manche Autoren eine Steigerung der Funktion des RES durch Follikelhormon (Haendel[11], Kinoshita[12], Fukuoka[16], Fujii[22]), Aber Uchino[21] und Fujii[22] behaupteten eine Herabsetzung durch Corpusluteumhormon, im Gegensatz dazu berichtete Kuga[23] über deren Steigerung. Nach der Pankreasexstirpation beobachteten Leites und Riabow[14], dass die Funktion des RES unverändert bleibt. Aber viele Autoren berichteten über eine Funktionssteigerung des RES durch Insulin (Goldzieher[7], Leites[14], Haendel[11], Samaras[24]), umgekehrt Saxl und Donath[15] über eine Herabsetzung, während Kinoshita[12] und Uchino[21] betonten, dass die Funktion des RES durch Insulin in grosser Menge abnimmt, aber durch nachfolgende Insulininjektion in kleiner Menge wieder zunimmt.

Aus unseren Versuchsergebnissen wird klar, dass verschiedene Hormone wie Hypophorin, Pituitrin, Syncorta, Rindex, Thyroxin, Praehormon, Synahorin, Rohormon, Progenin, Ovoglandol, Amolysin in kleiner Menge, Insulin und Padutin, nicht aber Acthar, Amolysin in grosser Menge und Perandren, die Funktion des RES steigern, und dass die Leberzellenfunktion durch Acthar, Praehormon, Synahorin, Rohormon, Progenin und Amolysin dagegen gereizt wird.

Im Vergleich mit obigen Hormonforschungen gibt es Untersuchungen über Vitaminwirkungen relativ wenig. Betreffs Vitamin A betonte Fujita[25] eine Funktionssteigerung des RES durch geeignete Dosierung, umgekehrt eine Herabsetzung durch Ueberdosierung. Aber Uotila und Simola[26] wiesen histologisch eine Funktionssteigerung des RES bei A-Avitaminose und deren Herabsetzung bei A-Hypervitaminose nach. Weiterhin bemerkte Tsurui[27] eine Funktionssteigerung des RES durch Vitamin B_1 sowie B_2, aber Morioka[27] stellte eine unveränderte Funktion fest, während Uotila[26] und Gaál[29] bei B_1-Hypervitaminose eine

Funktionssteigerung des RES bemerkten. Ausserdem teilten Kawaguchi[30], Tasaka[31] und Tsurui[27] eine Steigerung der Funktion des RES durch Vitamin C mit, aber Fukuoka[16] und Morioka[28] stellten eine unveränderte Funktion fest, während Uotila und Simola[26] eine Funktionssteigerung des RES bei C-Avitaminose bemerkten. Aber Horikawa[32] behauptete eine Herabsetzung der Funktion des RES durch Überdosierung von Vitamin D.

Aus unseren Versuchsergebnissen folgt, dass verschiedene Vitamine wie Vitadol, Metabolin, Bisulase, Aderoxin, Fresmin, Niamide, Folical, Redoxon, Ovoral, Juvela, Kativ und Hesperin die Funktion des RES steigern, jedes bis zu einem gewissen Grade, dagegen wird die Leberzellenfunktion nur durch Metabolin, Bisulase, Fresmin, Folical, Kativ und Hesperin gereizt.

Bisher ist keine Meinung über den Wirkungsunterschied zwischen Hormon und Vitamin auf die Funktion des RES veröffentlicht worden. Nach unseren Versuchsergebnissen wird die stimulierende Wirkung von Hypophorin auf die Funktion des RES durch Luminal gehemmt, dagegen wird die obige Wirkung von Vitadol durch Luminal ganz aufgehoben.

Daraus folgt also, dass Hypophorin die Funktion des RES direkt steigert, Vitadol dagegen mittelst des vegetativen Nervenzentrums die Funktion des RES stimuliert.

Zusammenfassung

Fassen wir die obigen Versuchsergebnisse zusammen, so gelangen wir zu folgendem Schluss:

1. Von den verschiedenen Hormonen verursachen Hypophorin, Pituitrin, Syncorta, Rindex, Thyroxin, Praehormon, Synahorin, Rohormon, Progenin, Ovoglandol, Amolysin in kleiner Menge, Insulin und Padutin, nicht aber Acthar, Amolysin in grosser Menge und Perandren eine Verminderung des Kongorotindex, dagegen steigt die Prothrombinzahl durch Acthar, Praehormon, Synahorin, Rohormon, Progenin und Amolysin an.

2. Verschiedene Vitamine wie Vitadol, Metabolin, Bisulase, Aderoxin, Fresmin, Niamide, Folical, Redoxon, Kativ und Hesperin setzen den Kongorotindex herab, jedes bis zu einem gewissen Grade, dagegen vermehrt sich die Prothrombinzahl durch Vitadol, Metabolin, Bisulase, Fresmin, Folical, Redoxon und Kativ, und vermindert sich durch Juvela, während sie durch Aderoxin, Niamide, Ovoral und Hesperin unverändert bleibt.

3. Die obenerwähnte Herabsetzung des Kongorotindex bei Hypophorin wird nicht durch Luminal gehemmt, dagegen wird die obige Wirkung von Vitadol durch Luminal ganz aufgehoben. Daraus lässt sich

schliessen, dass Hypophorin die Funktion des RES direkt steigern kann, im Gegensatz dazu Vitadol mittelst des vegetativen Nervenzentrums die Funktion des RES stimuliert.

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Experimentelle Studien über die Funktion des reticuloendothelialen Systems

VI. Mitteilung

Einfluss verschiedener leberstärkender sowie entgiftender Mittel auf die Funktion des RES und der Leber

Von

Shoichi Yamagata, Toshio Aratani, Kiyomi Miura und
(山形 敞一) (荒谷 利夫) (三浦 清美)

Kisao Otomo
(大友 喜佐雄)

Aus der Med. Klinik von Prof. Dr. T. Kurokawa, Tohoku Universität, Sendai

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Es ist schon bekannt, dass Glukose, mehrere Hormone und Vitamine als leberstärkende Mittel von verschiedenen Autoren aufgezählt worden sind. Aber wir haben in der früheren Mitteilung[1] dieser Arbeit beobachtet, dass der grösste Teil dieser leberstärkenden Mittel mehr die Funktion des RES als die Leberzellenfunktion steigern kann. Dagegen gehen die Meinungen der Autoren betreffs des Wirkungsmechanismus der entgiftenden Mittel auseinander; einige Autoren halten die Leberfunktion für wichtig, während andere Gewicht auf die Funktion des RES legen.

Unsere diesbezügliche Untersuchung zielt darauf, zu erfahren, wie das leberstärkende sowie entgiftende Mittel auf die Funktion des RES und der Leber, sowohl beim gesunden als auch geschädigten Zustand der Leber, einwirkt. Die Auswahl der Versuchstiere sowie die Methodik war die gleiche wie bei der früheren Mitteilung.[1]

Versuchsergebnisse

1. Einfluss verschiedener leberstärkender Mittel

Wenn man 25% Glukose in Menge von 10 ccm pro kg oder Kativ (Vitamin K) in Menge von 10 mg pro kg in die Vene des gesunden Kaninchens injiziert, so vermindert sich, wie in Tabelle I zeigt, der Kongorotindex, dagegen vermehrt sich die Prothrombinzahl. Aber die Injektion von DL-oder L-Methionin in Menge von 20 mg pro kg verursacht eine Vermehrung des Kongorotindex, während die Prothrombinzahl unverändert bleibt. Weiterhin vermindert sich durch DL-Methionin in Menge von 4 mg pro kg der Kongorotindex in leichtem Grade, doch sofort nach der Injektion von L-Methionin in Menge von 50 mg pro kg

TABELLE I

Einfluss der leberstärkenden Mittel

	Dose (pro kg)		Nr. des Kaninchens	Vor. Inj.	Zeit nach Injektion (St.) 1	3	5
Glucose	25% 10 ccm i.v.	K-index	1	63.5	66.5	69.3	80.0
			2	52.0	57.5	48.4	82.8
			Durchschn.	57.5	62.6	59.7	81.3
			Differenz		-6.7	-16.9	6.3
		P-zahl	1	1.00	1.09	1.07	1.02
			2	1.00	1.07	1.01	1.01
			Durchschn.	1.00	1.08	1.04	1.02
			Differenz		0.09	0.07	0.04
Kativ	10 mg i.v.	K-index	1	63.3	70.5	69.7	76.8
			2	51.3	51.6	44.5	65.0
			Durchschn.	56.8	60.7	55.2	72.1
			Differenz		-7.9	-11.4	-2.2
		P-zahl	1	1.00	1.03	1.12	1.17
			2	1.00	1.09	1.09	1.06
			Durchschn.	1.00	1.06	1.11	1.12
			Differenz		0.07	0.14	0.14
DL-Methionin	4 mg s.c.	K-index	1	58.3	57.2	68.2	68.2
			2	51.6	68.0	66.7	70.0
			Durchschn.	55.0	62.6	67.3	69.2
			Differenz		-4.2	-6.8	-3.3
		P-zahl	1	1.00	0.98	1.00	1.05
			2	1.00	1.00	1.00	0.93
			Durchschn.	1.00	0.99	1.00	0.99
			Differenz		0	0.03	0.01
	20 mg s.c.	K-index	1	64.4	64.0	70.8	56.2
			2	56.2	50.0	66.0	62.5
			Durchschn.	60.3	57.2	68.7	58.7
			Differenz		-14.9	-10.7	-19.1
		P-zahl	1	1.00	1.00	1.00	1.05
			2	1.00	1.00	0.99	0.99
			Durchschn.	1.00	1.00	1.00	1.00
			Differenz		0.01	0.03	0.02
L-Methionin	20 mg s.c.	K-index	1	68.8	54.5	72.8	72.1
			2	65.6	47.3	47.9	48.3
			Durchschn.	67.3	50.9	61.3	62.5
			Differenz		-20.9	-17.0	-21.3
		P-zahl	1	1.00	1.01	1.03	1.03
			2	1.00	1.01	1.02	1.05
			Durchschn.	1.00	1.01	1.03	1.04
			Differenz		0.01	0.01	0
	50 mg s.c.	K-index	1	57.2	80.0	58.3	57.1
			2	50.0	65.8	60.7	57.6
			Durchschn.	53.4	72.8	59.4	57.2
			Differenz		13.7	-5.0	-12.7
		P-zahl	1	1.00	1.06	0.99	1.02
			2	1.00	1.04	1.02	1.11
			Durchschn.	1.00	1.05	1.01	1.07
			Differenz		0.05	-0.01	0.03

vermehrt sich der Kongorotindex, während die Prothrombinzahl bei dem ersteren unbeeinflusst und bei dem letzteren gesteigert wird.

Daraus erhellt, dass von den leberstärkenden Mitteln Glukose und Kativ eine Steigerung der Funktion des RES und der Leberzellen hervorruft, dagegen ist die stimulierende Wirkung von Methionin auf die Leberzellenfunktion nicht so kräftig.

2. *Einfluss verschiedener entgiftender Mittel*

Wie aus Tabelle II und III zu ersehen ist, ruft die Injektion von 10% Dechycol (Dehydrocholsaures Natrium) in Menge von 20 mg pro kg, 0.5% Choloytardin (Taurocholsaures Natrium) in Menge von 5 mg pro kg, Gedox (Gallenpräparat) in Menge von 1 ccm pro kg, Guronsan (Glucuronsäure) in Menge von 50 mg pro kg oder 50% Hypon (Natriumthiosulfat) in Menge von 0.5 g pro kg eine Verminderung des Kongorotindex hervor, dagegen bleibt die Prothrombinzahl unverändert. Weiterhin vermindert sich durch Gluthation in Menge von 2 mg pro kg der Kongorotindex und vermehrt sich die Prothrombinzahl. Im Gegensatz

TABELLE II

Einfluss der Gallenpräparate

	Dose (pro kg)		Nr. des Kaninchens	Vor. Inj.	Zeit nach Injektion (St.)		
					1	3	5
Dechycol	2% 1 ccm i.v.	K-index	1	59.5	66.6	66.5	56.3
			2	39.5	34.7	34.5	48.0
			Durchschn.	50.6	52.3	53.2	53.5
			Differenz		-10.1	-16.7	-14.6
		P-zahl	1	1.00	1.05	1.02	1.00
			2	1.00	0.98	0.98	1.00
			Durchschn.	1.00	1.02	1.00	1.00
			Differenz		0.03	0.03	0.02
Choloytardin	5.5% 1 ccm s.c.	K-index	1	49.6	43.3	62.0	74.0
			2	40.6	35.1	46.3	45.5
			Durchschn.	45.4	39.7	54.3	59.8
			Differenz		-17.5	-10.2	-3.1
		P-zahl	1	1.00	1.01	1.11	1.08
			2	1.00	1.00	1.00	1.07
			Durchschn.	1.00	1.01	1.06	1.08
			Differenz		0	0.03	0.06
Gedox	1 ccm i.v.	K-index	1	60.0	62.5	65.2	69.5
			2	57.8	48.7	51.3	56.8
			Durchschn.	59.1	55.8	60.0	64.8
			Differenz		-6.5	-3.4	2.0
		P-zahl	1	1.00	1.00	1.02	1.02
			2	1.00	1.02	0.99	0.99
			Durchschn.	1.00	1.01	1.01	1.01
			Differenz		0	-0.02	-0.02

TABELLE III

Einfluss der entgiftenden Mittel

	Dose (pro kg)		Nr. des Kaninchens	Vor Inj.	Zeit nach Injektion (St.)		
					1	3	5
Guronsan	20 mg s.c.	K-index	1	54.8	58.7	68.6	62.5
			2	53.8	54.2	75.7	80.7
			Durchschn.	54.4	56.6	71.4	70.6
			Differenz		-2.3	6.0	-0.3
		P-zahl	1	1.00	1.00	1.02	1.03
			2	1.00	0.98	0.96	0.97
			Durchschn.	1.00	0.99	0.99	1.00
			Differenz		-0.01	-0.03	-0.04
	50 mg s.c.	K-index	1	66.7	41.4	62.5	56.3
			2	25.3	29.1	46.4	54.3
			Durchschn.	42.3	32.3	54.6	55.4
			Differenz		-14.5	1.3	-3.4
		P-zahl	1	1.00	1.04	1.01	1.04
			2	1.00	1.00	0.99	1.00
			Durchschn.	1.00	1.02	1.00	1.02
			Differenz		0.02	-0.02	-0.02
Gluthation	2 mg s.c.	K-index	1	52.4	58.5	59.8	54.3
			2	45.8	41.7	59.4	56.8
			Durchschn.	48.5	49.2	59.2	55.7
			Differenz		-11.1	-8.4	-10.3
		P-zahl	1	1.00	1.08	1.08	1.19
			2	1.00	1.03	1.07	1.11
			Durchschn.	1.00	1.06	1.08	1.15
			Differenz		0.07	0.11	0.17
BAL	0.2 ccm i.m.	K-index	1	80.5	46.7	38.5	79.8
			2	58.2	32.8	52.1	43.5
			Durchschn.	69.1	39.5	45.3	58.8
			Differenz		-32.8	-28.1	-14.0
		P-zahl	1	1.00	0.99	0.99	0.95
			2	1.00	0.98	0.99	0.96
			Durchschn.	1.00	0.99	0.99	0.96
			Differenz		-0.02	-0.05	-0.07
Hypon	50% 1 ccm i.v.	K-index	1	53.2	56.5	66.7	50.0
			2	39.6	39.3	29.9	19.1
			Durchschn.	48.2	51.1	55.3	45.6
			Differenz		-10.9	-5.6	-13.6
		P-zahl	1	1.00	1.00	0.99	1.03
			2	1.00	1.01	0.97	1.01
			Durchschn.	1.00	1.01	0.98	1.02
			Differenz		0.01	-0.01	0

dazu verursacht die Injektion von BAL (Dithiol) in Menge von 0.2 ccm pro kg eine deutliche Verminderung sowohl des Kongorotindex als auch der Prothrombinzahl.

Was die entgiftenden Hormone betrifft, so vermindert sich, wie Tabelle IV zeigt, der Kongorotindex durch Yakriton (das entgiftende Leberhormon) in Menge von 0.5 EH pro kg, Retio A (das Autohormon der reticuloendothelialen Zellen, hauptsächlich aus Milz und Lymph drüsen) oder Retio B (dasselbe hauptsächlich aus Knochenmark und Leber) in Menge von 1 EH pro kg, während die Prothrombinzahl durch Yakriton gesteigert, aber durch Retio A und B unbeeinflusst wird.

TABELLE IV

Einfluss der entgiftenden Hormone

	Dose (pro kg)		Nr. des Kaninchens	Vor Inj.	Zeit nach Injektion (St.)		
					1	3	5
Yakriton	0.5 EH s.c.	K-index	1	55.8	51.4	52.0	52.0
			2	53.3	49.3	60.3	49.0
			Durchschn.	54.8	50.3	55.8	50.8
			Differenz		-7.7	-3.3	-7.7
		P-zahl	1		1.00	1.03	1.04	1.06
			2		1.00	1.01	1.05	1.05
			Durchschn.		1.00	1.02	1.05	1.06
			Differenz			0.03	0.08	0.08
Retio A	1 EH s.c.	K-index	1		49.5	35.5	51.1	46.1
			2		42.7	41.6	43.7	50.0
			Durchschn.		44.3	38.2	47.4	47.9
			Differenz			-9.3	-1.1	-0.1
		P-zahl	1		1.00	0.99	1.02	0.98
			2		1.00	0.99	0.99	0.97
			Durchschn.		1.00	0.99	1.01	0.98
			Differenz			-0.02	-0.02	-0.05
Retio B	1 EH s.c.	K-index	1		68.2	65.8	61.5	74.8
			2		53.8	50.0	69.8	73.2
			Durchschn.		57.7	56.2	63.6	68.6
			Differenz			-12.3	-13.2	-6.4
		P-zahl	1		1.00	1.00	1.00	1.04
			2		1.00	1.03	1.00	1.02
			Durchschn.		1.00	1.02	1.00	1.03
			Differenz			0.01	-0.03	0

Deshalb ist es begreiflich, dass die Mehrzahl der entgiftenden Mittel die Funktion des RES, doch nicht immer die Leberzellenfunktion steigern können.

3. Einfluss der leberstärkenden und entgiftenden Mittel auf die geschädigte Leber.

Wenn man 25% Glukose in Menge von 10 ccm 5 Stunden nach totaler Choledochusligatur intravenös injiziert, so vermindert sich, wie in Tabelle V gezeigt wird, der Kongorotindex und vermehrt sich die Pro-

TABELLE V

Einfluss der leberstärkenden Mittel bei Choledochusligatur

		Nr. des Kaninchens	Vor Op.	5 St. nach Op.	Zeit nach Injektion (St.) 1	3	5
Choledochusligatur	K-index	1	74.7	77.8	75.0	88.3	93.3
		2	63.2	74.9	81.2	85.8	91.7
		Durchschn.	69.0	76.3	78.1	86.6	92.3
		Differenz			1.8	10.3	16.0
	P-zahl	1	1.00	0.92	1.00	1.00	1.00
		2	1.00	0.79	0.89	0.83	0.86
		Durchschn.	1.00	0.85	0.99	0.91	0.93
		Differenz		(1.00)	-0.06	-0.09	-0.07
Glukose nach Choledochusligatur	K-index	1	67.3	86.8	76.8	82.0	94.5
		2	43.5	95.0	88.8	81.2	94.2
		Durchschn.	55.8	89.9	81.6	81.7	94.3
		Differenz			-8.3	-8.2	4.4
	P-zahl	1	1.00	0.62	1.11	1.08	1.22
		2	1.00	0.66	1.10	1.12	1.12
		Durchschn.	1.00	0.64	1.11	1.10	1.17
		Differenz		(1.00)	0.17	0.19	0.24
L-Methionin nach Choledochusligatur	K-index	1	62.7	76.8	87.5	94.1	91.6
		2	55.1	83.4	93.8	94·1	57.2
		Durchschn.	58.8	79.6	90.4	94.1	72.5
		Differenz			9.0	4.2	-23.1
	P-zahl	1	1.00	0.90	1.01	0.97	0.90
		2	1.00	0.75	1.01	0.94	0.79
		Durchschn.	1.00	0.83	1.01	0.96	0.85
		Differenz		(1.00)	0.07	0.05	-0.08
Kativ nach Choledochusligatur	K-index	1	59.8	77.2	80.6	88.8	86.8
		2	58.3	80.0	84.4	91.1	94.5
		Durchschn.	59.2	78.2	82.3	89.7	90.1
		Differenz			2.3	1.2	-4.1
	P-zahl	1	1.00	0.80	1.03	0.97	0.96
		2	1.00	0.85	1.01	0.90	0.98
		Durchschn.	1.00	0.83	1.02	0.94	0.97
		Differenz		(1.00)	0.08	0.03	0.04
Hypon nach Choledochusligatur	K-index	1	47.7	83.8	88.7	89.0	84.6
		2	45.5	82.7	93.8	94.4	81.2
		Durchschn.	46.7	83.3	90.8	91.7	83.0
		Differenz			5.7	-1.9	-16.3
	P-zahl	1	1.00	0.75	1.05	1.04	1.04
		2	1.00	0.88	0.95	0.90	0.88
		Durchschn.	1.00	0.82	1.00	0.97	0.96
		Differenz		(1.00)	0.06	0.06	0.03

thrombinzahl. Gegenüber der Glukoseinjektion, verursacht die Injektion von L-Methionin in Menge von 20 mg pro kg, Kativ in Menge von 10 mg pro kg oder 50% Hypon in Menge von 0.5 g, eine Vermehrung

des Kongorotindex mit nachfolgender Herabsetzung und ebenso eine Vermehrung der Prothrombinzahl. Aber bei der Injektion von Glukose, L-Methionin oder Hypon 5 Stunden nach Chloroforminjektion im Menge von 0.25 ccm pro kg, vermindert sich, wie man aus Tabelle VI ersieht, der Kongorotindex, dagegen findet eine Vermehrung der Prothrombinzahl nicht so deutlich statt. Wie aus Tabelle VII ersichtlich ist, ruft die Injektion von Glukose, L-Methionin oder Kativ 5 Stunden nach 2 Leberlappenresektionen eine Verminderung des Kongorotindex sowie eine Vermehrung der Prothrombinzahl hervor.

TABELLE VI

Einfluss der leberstärkenden Mittel bei Chloroformintoxikation

		Nr. des Kaninchens	Vor Intox.	5 St. nach Intox.	Zeit nach Injektion (St.)		
					1	3	5
Chloroform-intoxikation	K-index	1	51.8	52.5	64.2	70.7	77.3
		2	47.5	50.7	62.2	68.6	65.2
		Durchschn.	49.6	51.6	62.8	69.6	70.8
		Differenz			11.2	18.0	19.2
	P-zahl	1	1.00	1.02	0.94	0.92	0.85
		2	1.00	0.90	0.93	0.94	0.83
		Durchschn.	1.00	0.96	0.94	0.93	0.84
		Differenz		(1.00)	-0.06	-0.07	-0.16
Glukose nach Chloroform-intoxikation	K-index	1	50.0	64.7	68.6	75.0	75.0
		2	50.0	60.0	75.0	63.6	82.3
		Durchschn.	50.0	61.7	72.2	67.6	74.3
		Differenz			-0.7	-12.1	-6.6
	P-zahl	1	1.00	0.92	1.01	0.88	0.68
		2	1.00	0.92	0.98	0.99	0.88
		Durchschn.	1.00	0.92	1.00	0.94	0.78
		Differenz		(1.00)	0.06	0.01	-0.06
L-Methionin nach Chloroform-intoxikation	K-index	1	78.8	67.7	56.7	78.3	72.3
		2	50.0	64.7	74.0	70.2	53.4
		Durchschn.	65.2	66.0	65.2	71.0	63.3
		Differenz			-12.0	-13.0	-21.9
	P-zahl	1	1.00	0.88	0.99	0.95	0.78
		2	1.00	0.90	0.99	0.87	0.77
		Durchschn.	1.00	0.89	0.99	0.91	0.77
		Differenz		(1.00)	0.05	-0.02	-0.07
Hypon nach Chloroform-intoxikation	K-index	1	61.6	67.3	62.8	62.0	80.0
		2	56.8	55.0	53.2	56.3	70.6
		Durchschn.	59.3	60.6	57.3	59.2	75.7
		Differenz			-14.5	-19.4	-4.1
	P-zahl	1	1.00	0.90	0.97	1.03	0.86
		2	1.00	0.99	0.97	0.95	0.88
		Durchschn.	1.00	0.95	0.97	0.99	0.87
		Differenz		(1.00)	0.03	0.06	0.03

TABELLE VII

Einfluss der leberstärkenden Mittel bei 2 Leberlappenresektionen

		Nr. des Kaninchens	Vor Op.	5 St. nach Op.	Zeit nach Injektion (St.) 1	3	5
2 Leberlappenresektion	K-index	1	61.6	77.6	77.2	83.3	81.8
		2	58.7	77.0	84.8	90.8	87.3
		3	52.7	62.5	77.0	83.3	77.8
		Durchschn.	57.2	71.8	79.8	86.3	82.5
		Differenz			8.0	14.5	10.7
	P-zahl	1	1.00	0.94	0.98	0.95	0.96
		2	1.00	0.96	0.98	0.95	0.94
		3	1.00	0.89	0.96	0.94	0.91
		Durchschn.	1.00	0.93	0.97	0·95	0.94
		Differenz		(1.00)	-0.03	-0.05	-0.06
Glukose nach 2 Leberlappenresektion	K-index	1	50.0	80.0	78.0	78.6	83.1
		2	48.5	84.0	82.5	86.8	84.6
		Durchschn.	49.3	82.0	80.2	82.2	83.7
		Differenz			-6.2	-14.3	-9.3
	P-zahl	1	1.00	0.95	1.02	1.08	1.11
		2	1.00	0.98	1.00	1.07	1.05
		Durchschn.	1.00	0.97	1.01	1.08	1.08
		Differenz		(1.00)	0.04	0.13	0.14
L-Methionin nach 2 Leberlappenresektion	K-index	1	60.1	78.2	82.2	78.8	86.7
		2	58.2	83.3	78.4	69.2	81.7
		Durchschn.	59.1	81.1	80.1	73.3	83.7
		Differenz			-9.1	-22.3	-8.1
	P-zahl	1	1.00	0.93	1.07	1.05	1.05
		2	1.00	0.95	1.05	1.03	1.02
		Durchschn.	1.00	0.94	1.06	1.04	1.04
		Differenz		(1.00)	0.09	0.09	0.10
Kativ nach 2 Leberlappenresektion	K-index	1	50.6	80.2	73.2	74.8	80.0
		2	49.0	76.3	77.0	81.8	89.0
		Durchschn.	49.7	77.2	75.3	78.4	84.6
		Differenz			-9.9	-13.3	-3.3
	P-zahl	1	1.00	0.95	0.92	0.94	1.01
		2	1.00	0.93	0.96	0.94	0.99
		Durchschn.	1.00	0.94	0.94	0.94	1.00
		Differenz		(1.00)	-0.03	-0.01	0.06

Aus den obenerwähnten Versuchen ist es klar geworden, dass die obigen leberstärkenden und entgiftenden Mittel die Funktion des RES auch bei Leberschädigungen steigern, aber die Leberzellenfunktion nur bei leichtgradig geschädigter Leber stimulieren können.

4. Einfluss des Luminals auf die Wirkung der leberstärkenden oder entgiftenden Mittel

Wie aus Tabelle VIII zu sehen ist, wird die schon erwähnte Verminderung des Kongorotindex nach der Injektion von Glukose, Yakriton

TABELLE VIII

Einfluss des Luminals auf die Wirkung der leberstärkenden sowie entgiftenden Mittel

	Dose (pro kg)		Nr. des Kaninchens	Vor Inj.	Zeit nach Injektion (St.) 1	3	5
Luminal	30 mg i.m.	K-index	1	50.0	55.6	68.0	65.1
			2	45.8	70.0	66.7	65.0
			Durchschn.	47.8	63.0	67.3	65.5
			Differenz		13.0	15.2	14.0
		P-zahl	1	1.00	1.00	1.00	0.96
			2	1.00	1.00	0.99	0.92
			Durchschn.	1.00	1.00	1.00	0.94
			Differedz		0	0.01	-0.07
Glukose	25% 10 ccm i.v.	K-index	1	63.5	66.5	69.3	80.0
			2	52.0	57.5	48.4	82.8
			Durchschn.	57.5	62.6	59.7	81.3
			Differenz		-6.7	-16.9	6.3
		P-zahl	1	1.00	1.09	1.07	1.02
			2	1.00	1.07	1.01	1.01
			Durchschn.	1.00	1.08	1.04	1.02
			Differenz		0.09	0.07	0.04
Glukose nach Luminal	Glucose 25% 10 ccm i.v. Luminal 30 mg i.m.	K-index	1	62.0	69.1	78.2	74.9
			2	39.1	77.4	70.0	70.0
			Durchschn.	50.8	72.8	74.8	72.8
			Differenz		10.2	4.9	4.5
		P-zahl	1	1.00	0.92	0.93	0.87
			2	1.00	0.88	0.90	0.92
			Durchschn.	1.00	0.90	0.92	0.90
			Differenz		-0.09	-0.05	-0.08
Yakriton	0.5 EH s.c.	K-index	1	55.8	51.4	52.0	52.0
			2	53.3	49.3	60.3	49.0
			Durchschn.	54.8	50.3	55.8	50.8
			Differenz		-7.7	-3.3	-7.7
		P-zahl	1	1.00	1.03	1.04	1.06
			2	1.00	1.01	1.05	1.05
			Durchschn.	1.00	1.02	1.05	1.06
			Differenz		0.03	0.08	0.08
Yakriton nach Luminal	Yakriton 0.5 EH s.c. Luminal 30 mg i.m.	K-index	1	45.4	69.8	73.9	88.4
			2	41.7	78.6	67.3	86.7
			Durchschn.	43.3	74.2	67.1	86.9
			Differenz		19.1	4.7	26.1
		P-zahl	1	1.00	1.03	1.11	1.10
			2	1.00	1.02	1.02	1.05
			Durchschn.	1.00	1.03	1.07	1.08
			Differenz		0.04	0.10	0.10
Hypon	50% 1 ccm i.v.	K-index	1	53.2	56.5	66.7	50.0
			2	39.6	39.3	29.9	19.1
			Durchschn.	48.2	51.1	55.3	45.6
			Differenz		-10.9	-5.6	-13.6
		P-zahl	1	1.00	1.01	0.97	1.01
			2	1.00	1.00	0.99	1.03
			Durchschn.	1.00	1.01	0.98	1.02
			Differenz		0.01	-0.01	0
Hypon nach Luminal	Hypon 50% 1ccm i.v. Luminal 30 mg i.m.	K-index	1	41.9	50.3	70.8	66.7
			2	35.1	43.6	63.2	45.7
			Durcnschn.	38.7	49.1	67.2	56.7
			Differenz		-5.6	15.6	7.6
		P-zahl	1	1.00	1.03	0.96	0.98
			2	1.00	1.00	0.99	0.97
			Durchschn.	1.00	1.01	0.98	0.98
			Differenz		0	-0.05	-0.05

und Hypon durch Luminal fast ganz gehemmt, dagegen wird eine Vermehrung der Prothrombinzahl bei Yakritoninjektion durch Luminal nicht gehemmt.

Daraus ist klar zu ersehen, dass Glukose, Yakriton und Hypon vermittelst des vegetativen Nervenzentrums die Funktion des RES stimulieren.

Diskussion

Seit langem ist es allgemein anerkannt, dass die Leber ein Hauptorgan der Entgiftung darstellt (Matsuo[2]). Im Gegensatz dazu betonen Haendel,[3] Masuhara[4] und Tokumitsu,[5] dass das RES sich an der Entgiftung beteiligt. Nach Matsuo[6] werden Glukose, Thyroxin, Lecithin und Choleretica leberstärkende Mittel genannt. Aber wir beobachteten in der früheren Mitteilung,[1] dass Glukose und Kativ die Funktion des RES sowie der Leberzellen, dagegen Thyroxin und Lecithin nur die Funktion des RES steigern. Bezüglich des von Tucker und Eckstein[7] als lipotroper Faktor erkannten Methionins, betonten Morrison,[8] Beams und Endicott[9] seine Heilwirkung für Lebercirrhose. Seitdem ist es allgemein anerkannt, dass Methionin eine leberstärkende Wirkung hat (Kitani[10]).

Aus unseren Versuchsergebnissen über leberstärkende Mittel ist klar geworden, dass Glukose und Kativ die Funktion des RES und der Leberzellen steigern, Methionin in geeigneter Menge dagegen wohl die Funktion des RES steigert, während seine Wirkung auf die Leberzellenfunktion nicht so kräftig ist.

In Bezug auf entgiftende Mittel fanden Matsuo und seine Schüler[6] eine Steigerung der Leberfunktion durch Choleretica; besonders betonte Matsuoka,[11] dass Taurocholsaures Natrium in geeigneter Menge die Farbstoffausscheidungsfunktion der Leber steigert. Im Gegensatz dazu fand Iimuro,[12] dass eine grosse Dose von Decholan die obige Leberfunktion hemmt. Aber Kawamoto,[13] beobachtete, dass die Entgiftungs-sowie Farbstoffausscheidungsfunktion der Leber durch Dehydrocholsäure gesteigert wird, während die obige Funktion durch Taurocholsäure teils gesteigert, teils geschädigt wird. Weiterhin bemerkte Tasaka[14] eine Steigerung der Farbstoffausscheidungs-sowie Entgiftungsfunktion der Leber durch Glucuronsäure und betonte Binet[15] die Entgiftungswirkung von Gluthation. Auch das von Peter[16] als BAL (British Anti-Lewsite) genannte Dithiol empfahlen Sulzburger und Beer[17] als entgiftendes Mittel. Betreffs des Natriumthiosulfates bemerkten Kabelik,[18] Takasugi usw.[19] eine Steigerung der Funktion der Entgiftungswirkung der Leber, aber Kawaguchi[20] berichtete über eine Steigerung der Funktion des RES. Weiterhin fand Sato[21] den entgiftenden Effekt von Yakriton und Miyakawa[22] denjenigen von Retio A und B.

Aus den obenerwähnten Versuchsergebnissen ist ersichtlich, dass solche entgiftenden Mittel wie Dechycol, Choloytardin, Gedox, Guronsan, BAL, Hypon, Retio A und B, nur die Funktion des RES bis zu einem gewissen Grade steigern, dagegen wird die Leberzellenfunktion nur durch Gluthation und Yakriton gesteigert.

Es ist sehr interessant, zu erfahren, wie diese leberstärkenden und entgiftenden Mittel auf die geschädigte Leber einwirken. Nach unserer früheren Mitteilung[23] ist die Leberschädigung durch totale Choledochusligatur äusserst hochgradig, aber diejenige durch 2 Leberlappenresektionen am leichtesten.

Aus unseren diesbezüglichen Versuchsergebnissen ist ersichtlich, dass Glukose die Funktion des RES und der Leberzellen bei den obigen Leberschädigungen steigern kann, aber andere Mittel, wie Kativ, Methionin und Hypon, die Funktion des RES auch bei verschiedenen Leberschädigungen stimulieren, während die obigen Mittel mehr oder weniger günstig auf die Leberzellenfunktion einwirken.

Infolgedessen ist die Vermutung ganz wahrscheinlich, dass sich die Funktion des RES an der Entgiftungsfunktion inniger beteiligt als die Leberzellenfunktion.

Wie aus unserer früheren Mitteilung[23] zu ersehen, wird eine Verminderung des Kongorotindex bei Hypophorin durch Luminal nicht gehemmt, dagegen wird die obige Wirkung bei Vitadol durch Luminal ganz aufgehoben. In ähnlicher Weise wie bei Vitadol wird die stimulierende Wirkung von Glukose, Yakriton und Hypon auf die Funktion des RES durch Luminal fast ganz gehemmt.

Daraus wird klar, dass die obenerwähnten leberstärkenden sowie entgiftenden Mittel vermittelst des vegetativen Nervenzentrums die Funktion des RES stimulieren.

Zusammenfassung

Fassen wir die obigen Versuchsergebnisse zusammen, so gelangen wir zu folgendem Schluss:

1. Von den verschiedenen leberstärkenden Mitteln vermindert sich der Kongorotindex durch Glukose, Kativ und Methionin in geeigneter Menge, dagegen vermehrt sich die Prothrombinzahl durch Glukose und Kativ, während sie durch Methionin unbeeinflusst bleibt.

2. Von den verschiedenen entgiftenden Mitteln verursachen Gluthation und Yakriton eine Verminderung des Kongorotindex und eine Vermehrung der Prothrombinzahl, aber durch andere Mittel, wie Dechycol, Choloytardin, Gedox, Guronsan, BAL, Hypon, Retio A und B, vermindert sich der Kongorotindex, während die Prothrombinzahl dadurch unverändert bleibt.

3. Bei totaler Choledochusligatur ruft Glukose eine Verminderung des Kongorotindex sowie eine Vermehrung der Prothrombinzahl hervor, aber Methionin, Kativ und Hypon am Anfang eine Steigerung des Kongorotindex, und nachher seine Verminderung. Weiterhin vermindert sich bei Chloroformintoxikation der Kongorotindex durch Glukose, Methionin und Hypon, wobei eine Vermehrung der Prothrombinzahl nicht so deutlich ist. Aber bei 2 Leberlappenresektionen verursacht Glukose, Methionin und Kativ eine Verminderung des Kongorotindex und eine Vermehrung der Prothrombinzahl.

4. Deshalb ist die Vermutung ganz wahrscheinlich, dass sich die Funktion des RES an der entgiftenden Wirkung inniger beteiligt als die Leberzellenfunktion.

5. Die obenerwähnte Herabsetzung des Kongorotindex bei der Injektion von Glukose, Yakriton und Hypon wird durch Luminal fast ganz gehemmt. Also ist klar, dass diese leberstärkenden sowie entgiftenden Mittel vermittelst des vegetativen Nervenzentrums die Funktion des RES stimulieren.

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Experimentelle Studien über die Funktion des reticuloendothelialen Systems

VII. Mitteilung

Einfluss des Shwartzmanschen Phänomens in der Leber auf die Funktion des RES und der Leber

Von

Shoichi Yamagata, Kiyomi Miura, Kisao Otomo und
(山形 敞一) (三浦 清美) (大友 喜佐雄)
Keiichi Takahashi
(高橋 惠一)

Aus der Med. Klinik von Prof. Dr. T. Kurokawa, Tohoku Universität, Sendai

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Im Jahre 1928 entdeckte Schwartzman[1] eine neue lokale Hautreaktivität für verschiedene bakterielle Kulturfiltrate und diese Erscheinung wurde Schwartzmansches Phänomen genannt. Darnach beobachteten manche Fachleute gleichartige Veränderungen in anderen Organgeweben und diskutierten ihre ätiologische Bedeutung.

Wir haben die vorliegende Untersuchung unternommen, um zu erfahren, welche Veränderungen in der Leber vor sich gehen und welcher Einfluss auf die Funktion des RES und der Leber ausgeübt wird.

Versuchsteil

Methodik

Als Schwartzmansches Filtrat wurde das Kulturfiltrat aus Colibacillen verwendet und die Auswahl der Versuchstiere sowie die Methodik war die gleiche wie bei der früheren Mitteilung.[2] Histologische Untersuchungen des Leberstückchens wurden mittels der Hämatoxylin-Eosinfärbung ausgeführt.

Versuchsergebnisse

1. Einfluss des Schwartzmanschen Filtrats

Wie aus Tabelle I zu sehen, ruft die intravenöse Injektion von Schwartzmanschem Filtrat in Verdünnung 1 : 2000 in Menge von 1 ccm pro kg eine Vermehrung des Kongorotindex und eine Verminderung der Prothrombinzahl hervor, aber in Verdünnung 1 : 5000 eine Verminderung des Kongorotindex und eine Vermehrung der Prothrombinzahl, während

TABELLE I

Einfluss der intravenösen Injektion von Schwartzmanschen Filtrat

Dose (pro kg)		Nr. des Kaninchens	Vor Inj.	Zeit nach Injektion (St.)					
				3	5	8	12	24	48
Sh-Filtrat (1 : 2000) 1 ccm i.v.	K-index	1	62.4	54.9	65.1	62.7	65.2	61.0	41.4
		2	54.8	52.8	67.9	68.1	72.6	56.2	54.4
		Durchschn.	58.2	53.6	67.2	66.3	69.7	57.9	49.0
		Differenz		-4.8	4.6	8.1	12.7	-0.4	-8.5
	P-zahl	1	1.00	1.00	1.02	1.01	0.97	0.97	1.05
		2	1.00	0.99	0.98	0.98	1.02	1.03	1.05
		Durchschn.	1.00	1.00	1.00	1.00	1.00	1.00	1.05
		Differenz		-0.02	-0.01	-0.05	-0.05	0	0.08
Sh-Filtrat (1 : 5000) 1 ccm i.v.	K-index	1	59.6	68.3	77.0	74.1	71.1	68.1	49.1
		2	56.5	49.2	50.0	56.0	57.3	61.4	48.9
		Durchschn.	58.1	57.8	62.4	66.5	63.8	64.1	48.8
		Differenz		-7.1	-7.2	-2.4	-1.8	-0.8	-17.1
	P-zahl	1	1.00	0.80	0.99	1.12	1.07	1.07	1.07
		2	1.00	1.00	0.95	0.94	0.98	1.05	1.06
		Durchschn.	1.00	0.90	0.97	1.03	1.03	1.06	1.07
		Differenz		-0.10	-0.02	0.02	0.03	0.06	0.07
Sh-Filtrat (1 : 10000) 1 ccm i.v.	K-index	1	63.2	60.6	70.5	63.0	71.8	67.4	56.7
		2	52.3	65.4	63.8	71.1	74.4	63.9	53.4
		Durchschn.	57.6	62.6	67.5	66.5	73.1	65.7	50.2
		Differenz		-1.8	-3.6	-1.1	8.0	1.3	-15.2
	P-zahl	1	1.00	1.07	0.94	0.96	0.87	0.83	0.89
		2	1.00	1.00	0.94	1.00	0.92	0.86	0.93
		Durchschn.	1.00	1.04	0.94	0.98	0.90	0.85	0.91
		Differenz		0.04	-0.05	-0.03	-0.10	-0.15	-0.09

in Verdünnung 1 : 10000 kein Einfluss auf die beiden zu bemerken ist.

Daher kommt es, dass Schwartzmansches Filtrat in hoher Konzentration die Funktion des RES und der Leberzellen herabsetzt, im Gegensatz dazu in dünner Konzentration die beiden steigert.

2. Einfluss des in der Leber entstandenen Schwartzmanschen Phänomens

Wenn man als Kontrollversuch physiologische Kochsalzlösung in Menge von 1 ccm pro kg in die Ohrvene injiziert, und zwar 24 Stunden nach intraportaler Injektion derselben Lösung in Menge von 0.1 ccm pro kg, so bleiben, wie Tabelle II zeigt, der Kongorotindex sowie die Prothrombinzahl fast unverändert. Aber bei intravenöser Injektion des Schwartzmanschen Filtrats in Verdünnung 1 : 20000 in Menge von 1 ccm pro kg 24 Stunden nach intraportaler Injektion desselben Filtrats in Verdünnung 1 : 10000 oder 1 : 20000 in Menge von 0.1 ccm pro kg vermehrt sich der Kongorotindex und die Prothrombinzahl vermindert sich, während sich die beiden vermehren bei einer Verdünnung von 1 : 5000 sowie 1 : 50000. Im Gegensatz dazu entsteht bei intrahepataler Injektion

Tabelle II

Einfluss des Schwartzmanschen Phänomens

Vorbereitung	Dose (pro kg)		Nr. des Kaninchens	Vor Inj.	24 St. nach Inj.	Zeit nach Shwartzmanschem Phänomen (St.) 3	5	8	12	24	48
0.9% NaCl-Lösg. 0.1 ccm i. port.	0.9% NaCl-Lösg. 1 ccm i.v.	K-index	1	53.3	57.6	55.0	64.8	50.0	53.3	52.5	44.3
			2	49.9	64.0	55.5	68.4	59.3	58.5	56.7	58.7
			3	41.6	51.1	59.5	63.3	57.8	60.8	57.0	53.1
			Durchschn.	47.3	56.8	57.0	65.5	55.5	57.3	55.4	52.0
			Differenz			0.2	8.7	-1.3	0.5	-1.4	-4.8
		P-zahl	1	1.00	1.04	1.00	1.05	1.05	1.01	1.01	1.10
			2	1.00	0.90	1.03	0.99	0.97	1.00	0.96	1.09
			3	1.00	1.01	1.03	1.05	1.04	1.00	0.97	1.04
			Durchschn.	1.00	0.98	1.02	1.03	1.02	1.00	0.98	1.08
			Differenz			0.02	0.03	0.02	0	-0.02	0.08
Sh-Filtrat (1 :10000) 0.1 ccm i. port.	Sh-Filtrat (1 :2000) 1 ccm i.v.	K-index	1	51.8	78.1	75.2	90.8	95.2	95.0	69.3	82.0
			2	40.8	62.8	65.2	81.3	85.0	77.7	88.3	60.8
			Durchschn.	46.0	71.4	70.6	87.2	90.8	87.8	76.7	73.0
			Differenz			-1.0	7.1	20.7	15.9	6.7	6.4
		P-zahl	1	1.00	0.99	0.83	0.91	0.89	0.90	1.03	1.02
			2	1.00	0.88	0.85	0.87	0.84	0.83	0.90	1.00
			Durchschn.	1.00	0.94	0.84	0.89	0.87	0.87	0.97	1.01
			Differenz			-0.18	-0.14	-0.15	-0.13	0.01	-0.07
Sh-Filtrat (1 :20000) 0.1 ccm i. port.	Sh-Filtrat (1 :2000) 1 ccm i.v.	K-index	1	68.8	65.3	77.5	75.7	83.3	71.4	71.1	68.8
			2	63.8	55.7	70.6	85.2	82.2	68.2	68.6	64.2
			Durchschn.	66.3	59.8	74.1	80.3	82.8	69.8	70.0	66.5
			Differenz			14.1	11.8	24.3	9.5	11.6	11.5
		P-zahl	1	1.00	0.97	0.87	0.87	1.00	1.00	0.93	0.96
			2	1.00	1.03	0.83	0.91	0.87	0.88	0.97	0.98
			Durchschn.	1.00	1.00	0.85	0.89	0.94	0.94	0.95	0.97
			Differenz			-0.17	-0.14	-0.08	-0.06	-0.03	-0.11
Sh-Filtrat (1 :50000) 0.1 ccm i. port.	Sh-Filtrat (1 :5000) 1 ccm i.v.	K-index	1	54.3	60.3	51.2	70.2	72.3	68.3	64.0	66.4
			2	51.4	65.8	56.1	63.6	81.0	68.5	62.5	54.3
			3	50.6	62.3	67.4	73.5	74.4	70.2	70.0	65.3
			Durchschn.	51.8	62.6	58.3	69.2	75.5	69.1	65.5	61.7
			Differenz			-4.5	-2.1	14.2	6.2	4.3	3.9
		P-zahl	1	1.00	0.86	1.24	1.02	1.02	1.11	1.12	1.17
			2	1.00	0.86	1.17	1.09	1.15	1.21	1.19	1.14
			3	1.00	0.87	1.21	1.17	1.19	1.20	1.08	1.18
			Durchschn.	1.00	0.86	1.21	1.09	1.12	1.17	1.13	1.16
			Differenz			0.19	0.07	0.10	0.17	0.15	0.08
Sh-Filtrat (1:50000) 0.1 ccm i. hepatal	Sh-Filtrat (1 :5000) 1 ccm i.v.	K-index	1	61.1	57.2	72.8	77.4	82.2	81.2	55.3	59.4
			2	51.2	54.8	62.6	69.7	88.2	78.2	74.2	72.6
			3	38.6	34.7	66.7	57.2	63.8	65.8	44.5	58.6
			Durchschn.	48.3	47.8	67.5	68.0	77.8	76.1	56.3	62.6
			Differenz			19.5	11.5	31.3	17.8	9.9	19.6
		P-zahl	1	1.00	0.95	0.99	0.99	1.07	1.02	1.01	1.02
			2	1.00	0.96	0.99	0.99	0.93	1.02	0.98	1.00
			3	1.00	1.08	0.86	0.85	0.86	0.92	0.93	0.96
			Durchschn.	1.00	1.00	0.93	0.92	0.95	0.98	0.97	0.99
			Differenz			-0.09	-0.11	-0.07	-0.02	-0.01	-0.09

eine Vermehrung des Kongorotindex und eine Verminderung der Prothrombinzahl.

Deshalb ist zu vermuten, dass sich die Funktion des RES und der Leberzellen vermindert bei dem in der Leber entstandenen Schwartzmanschen Phänomen.

3. Einfluss der leberstärkenden Mittel auf das in der Leber entstandene Schwartzmansche Phänomen

Tabelle III zeigt, dass im zuletztgenannten Versuche mit intrahepatal-

TABELLE III

Einfluss der leberstärkenden Mittel bei Leberschädigungen infolge von dem Schwartzmanschen Phänomen

	Dose (pro kg)		Nr. des Kaninchens	Vor Inj.	5 St. nach Sh.	Zeit nach Injektion (St.) 1	3	5
Shwartzmansches Phänomen	—	K-index	1	43.6	91.3	95.0	95.0	85.6
			2	40.4	80.0	80.0	83.4	88.8
			Durchschn.	42.1	84.3	86.7	89.3	87.3
			Differenz			2·4	4.9	3.0
		P-zahl	1	1.00	1.01	0.99	0.98	1.04
			2	1.00	0.95	1.02	0.97	1.01
			Durchschn.	1.00	0.98	1.01	0.98	1.03
			Differenz			0.01	-0.02	0.03
Glukose nach dem Shwartzmanschen Phänomen	25% 10 ccm i.v.	K-index	1	51.4	65.6	69.2	59.3	73.0
			2	41.9	52.7	48.3	72.3	74.3
			3	37.2	83.2	87.3	85.0	87.4
			Durchschn.	44.5	68.3	68.2	72.7	79.4
			Differenz			-2.5	-0.5	8.1
		P-zahl	1	1.00	0.84	1.12	1.09	1.17
			2	1.00	0.99	1.03	1.03	0.97
			3	1.00	1.03	0.97	0.94	0.94
			Durchschn.	1.00	0.95	1.04	1.02	1.03
			Differenz			0.03	0.04	0
L-Methionin nach dem Shwartzmanschen Phänomen	20 mg s.c.	K-index	1	56.3	76.3	68.2	82.9	76.1
			2	46.0	79.8	59.6	78.8	77.8
			Durchschn.	50.9	78.1	64.0	80.5	76.8
			Differenz			-16.5	-2.5	-4.3
		P-zahl	1	1.00	1.02	1.06	1.02	0.99
			2	1.00	0.95	0.99	1.01	1.02
			Durchschn.	1.00	0.99	1.03	1.02	1.01
			Differenz			0.02	0.04	-0.02
Kativ nach dem Shwartzmanschen Phnomen	10 mg i.v.	K-index	1	42.5	67.5	66.7	65.9	69.2
			2	41.6	62.2	52.7	69.0	68.1
			Durchschn.	42.0	64.8	59.7	67.2	68.7
			Differenz			-7.5	-2.5	0.9
		P-zahl	1	1.00	1.12	0.98	0.90	0.89
			2	1.00	0.98	1.07	1.07	1.02
			Durchschn.	1.00	1.10	1.03	0.99	0.96
			Differenz			0.02	0.01	-0.07

er Injektion der Kongorotindex sich leichtgradig vemehrt, aber die Prothrombinzahl fast unverändert bleibt, und zwar von der 5 bis zur 10 Stunde nach dem in der Leber entstandenen Schwartzmanschen Phänomen. Im diesen Falle verursacht die intravenöse Injektion von 25% Glukose im Menge von 10 ccm pro kg eine Verminderung des Kongorotindex sowie eine Vermehrung der Prothrombinzahl, und die subkutane Injektion von L-Methionin in Menge von 20 mg pro kg ruft eine deutliche Verminderung des Kongorotindex hervor, während die intravenöse Injektion von Kativ in Menge von 10 mg pro kg eine Verminderung des Kongorotindex sowie der Prothrombinzahl veranlasst.

Daraus erhellt, dass diese leberstärkenden Mittel auch bei dem in der Leber entstandenen Schwartzmanschen Phänomen guten Erfolg bringen.

4. Histologische Befunde bei dem in der Leber enstandenen Schwartzmanschen Phänomen

Tabelle IV zeigt, dass die histologischen Befunde der Kaninchenleber beim Kontrollversuche fast normal sind. Aber wir beobachteten beim obigen in der Leber entstandenen Schwartzmanschen Phänomen hepatitische Veränderungen wie Unordnung der Leberazini, periportale sowie intraazinöse entzündliche Zellinfiltration und Regeneration der Leber-

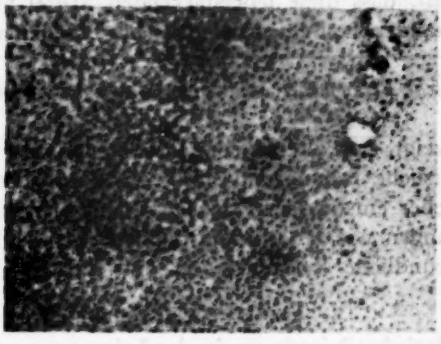
TABELLE IV

Histologische Befunde des in der Leber entstandenen Schwartzmanschen Phänomens

	Nr. des Kaninchens	periportale entz. Z-infiltration	intraazinöse entz. Z-infiltration	Unordnung der Leberazini	Degeneration der Leberzellen	Regeneration der Leberzellen	Sternzellen	Histologische Diagnose
Physiologische NaCl-lösung	1	−	−	−	−	−	normal	normal
	2	−	−	−	−	−	normal	normal
	3	−	−	+	−	−	normal	normal
Shwartzmansches Phänomen	1	+	+	++	±	+	fast OB	Hepatitis
	2	+	+	+	−	±	fast OB	Hepatitis
Glukose nach dem Sh-Phänomen	1	±	±	±	−	++	Hypertrophie, Wucherung	Regenerationserscheinung
	2	±	±	±	−	++	ditto	ditto
l-Methionin nach dem Sh-Phänomen	1	+	±	−	−	++	fast OB	ditto
	2	±	+	++	+	+	fast OB	Hepatitis
Kativ nach dem Sh-Phänomen	1	+	−	±	++	−	Hypertrophie, Wucherung	Regenerationserscheinung
	2	+	−	+	+	±	ditto	ditto

zellen. Durch intravenöse Injektion von 25% Glukose in Menge von 10 ccm pro kg bessern sich die Unordnung der Leberazini sowie die periportale entzündliche Zellinfiltration im obigen Falle, und kommen es deutliche Regeneration der Leberzellen und mässige Hypertrophie sowie Wucherung der Sternzellen zum Vorschein (Abb. 1–3). In ähnlicher Weise bessern sich die histologischen Befunde durch Injektion von L-Methionin in Menge von 20 mg pro kg oder Kativ in Menge von 10 mg pro kg anstatt der Glukoseinjektion (Abb. 4–5).

Abb. 1. Histologische Befunde der Leber bei intravenöser Injektion von physiologischer NaCl-lösung. Diagnose normal.



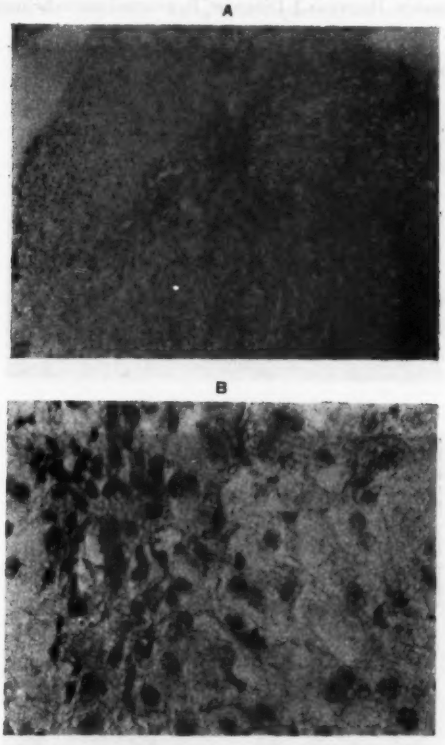
Diskussion

Das Shwartzmansche Phänomen beruht eigentlich auf vorübergehender gesteigerter lokaler Gewebsreaktion, wo eine Verminderung der Resistenz der lokalen Kapillarenwand durch erstmalige Injektion bakteriellen Kulturfiltrats hervorgerufen wird und eine Veränderung wie Hämorrhagie oder Nekrose durch zweite Injektion desselben Filtrats. Dieses Phänomen tritt nicht nur in der Haut hervor, sondern auch im Wurmfortsatz (Watanabe und Kimura[3]), in der Niere (Ouhchi und Yotsuyanagi[4]), im Pankreas (Tsuda[5], Kunitoki[6]) und sogar im Ohrlabyrinth (Tsuiki und Kamioka[7]).

Betreffs der Beziehungen des Schwartzmanschen Phänomens zur RES sowie der Leber, bemerkte Iwatsuki[8] eine Herabsetzung der Funktion des RES durch Shwartzmansches Filtrat in grosser Menge und ihre Steigerung durch dasselbe in kleiner Menge. Weiterhin berichtete Goshima[4] über eine auffallende Herabsetzung der Leberfunktion beim Shwartzmanschen Phänomen.

Durch unsere Versuchsergebnisse ist es klar geworden, dass die intravenöse Injektion des Schwartzmanschen Filtrats in Verdünnung 1 : 2000 die Funktion des RES herabsetzt, dasselbe in Verdünnung 1:50000

Abb. 2. Schwartzmansche Hepatitis. Diagnose Hepatitis.

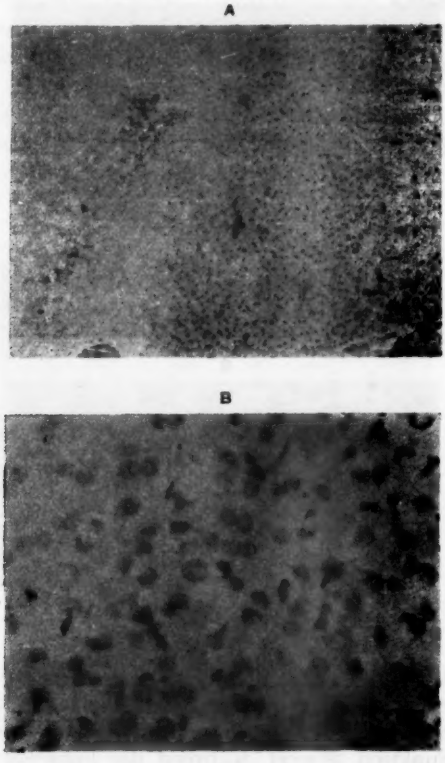


dagegen diese Funktion steigert, während die Funktion des RES durch Schwartzmansches Filtrat in Verdünnung 1 : 10000 fast unverändert bleibt. Dabei zeigt die Leberzellenfunktion die Neigung zur Abnahme. Bei dem in der Leber entstandenen Shwartzmanschen Phänomen durch intraportale oder intrahepatale Injektion des Shwartzmanschen Filtrats hat die Funktion des RES sowie der Leberzellen ebenfalls die Neigung zur Verminderung.

Schon in der früheren Mitteilung[10] dieser Untersuchung fanden wir, dass die Injektion von Glukose, L-Methionin und Kativ die Funktion des RES sowie der Leberzellen auch bei Leberschädigungen durch Choledochusligatur, Chloroformintoxikation und 2 Leberlappenresektionen steigert.

Aus unseren diesberzüglichen Versuchsergebnissen ist festzustellen, dass die Injektion von Glukose die Funktion des RES und der Leberzellen bei dem in der Leber entstandenen Shwartzmanschen Phänomen steigert, aber

Abb. 3. Intravenöse Injektion von 25% Glukose 10 ccm pro kg bei Schwartzmanscher Hepatitis. A Diagnose, B Regenerationserscheinung.



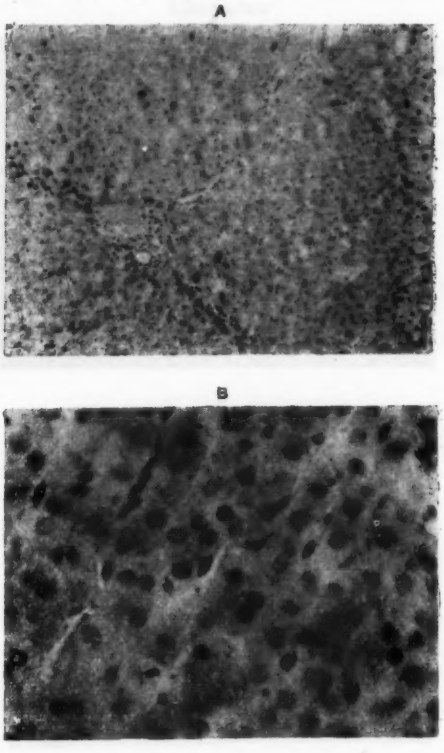
die Injektion von L-Methionin und Kativ dabei nur die Funktion des RES erhöhen kann. Weiterhin bestätigen wir durch obige histologische Untersuchungsergebnisse, dass das Schwartzmanshe Phänomen in der Leber hepatitische Veränderungen wie Unordnung der Leberazini, periportale sowie intraazinöse entzündliche Zellinfiltration und Regeneration der Leberzellen verursacht und die obigen Schädigungen sich durch Injektion von Glukose, L-Methionin und Kativ gebessert haben.

Infolgedessen ist es klar, dass diese leberstärkenden Mittel auch wirksam sind auf Hepatitis durch das in der Leber entstandene Shwartzmansche Phänomen.

Zusammenfassung

Aus den obigen Versuchsergebnissen können wir wie folgend schliessen:

Abb. 4. Subkutane Injektion von L-Methionin 20 mg pro kg bei Schwartzmanscher Hepatitis. Diagnose Regenerationserscheinung.

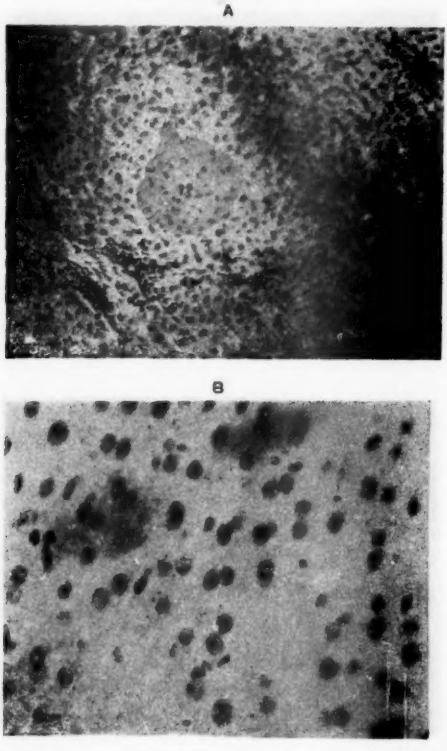


1. Die intravenöse Injektion des Shwartzmanschen Filtrats in Verdünnung 1 : 2000 in Menge von 1 ccm pro kg ruft eine leichte Vermehrung des Kongorotindex und eine Verminderung der Prothrombinzahl, aber dasselbe Filtrat in Verdünnung 1 : 5000 eine leichte Verminderung des Kongorotindex und eine Vermehrung der Prothrombinzahl hervor, während der Kongorotindex bei Injektion desselben Filtrats in Verdünnung 1 : 10000 fast unverändert bleibt.

2. Die intravenöse Injektion des Shwartzmanschen Filtrats in Verdünnung 1 : 2000 oder 1 : 5000 in Menge von 1 ccm pro kg 24 Stunden nach intraportaler oder intrahepataler Injektin desselben Filtrats in Verdünnung 1 : 10000 oder 1 : 50000 in Menge von 1 ccm pro kg ruft eine Vermehrung des Kongorotindex und eine Verminderung der Prothrombinzahl hervor, und zwar jedes in mehr oder weniger hohem Grade.

3. Die Injektion von Glukose 5 Stunden nach dem in der Leber

Abb. 5. Intravenöse Injektion von Kativ 10 mg pro kg bei Schwartmanscher Hepatitis. Diagnose Regenerationserscheinung.



entstandenen Shwartzmanschen Phänomen setzt den Kongorotindex herab und erhöht die Prothrombinzahl. Die Injektion von L-Methionin, anstatt der Glukose, setzt den Kongorotindex deutlich herab, aber die Prothrombinzahl bleibt dabei fast unverändert, während die Injektion von Kativ eine Verminderung des Kongorotindex sowie der Prothrombinzahl bemerken lässt.

4. Bei dem in der Leber entstandenen Shwartzmanschen Phänomen erscheinen hepatitische Veränderungen wie Unordnung der Leberazini, periportale sowie intraazinöse entzündliche Zellinfiltration und Regeneration der Leberzellen. Aber diese Veränderungen bessern sich durch Glukose, L-Methionin und Kativ, in entsprechend mehr oder weniger hohem Grade.

Infolgedessen unterliegt es keinem Zweifel, dass diese leberstärkenden Mittel auch wirksam sind auf Hepatitis durch das in der Leber entstandene

Shwartzmansche Phänomen.

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Experimentelle Studien über die Funktion des reticuloendothelialen Systems

VIII. Mitteilung

Einfluss der Chemotherapeutika, besonders der Sulfonamide und Antibiotika auf die Funktion des RES und der Leber

Von

Schoichi Yamagata, Toshio Aratani, Kiyomi Miura,
(山形 敞一) (荒谷 利夫) (三浦 清美)
Kisao Otomo und **Chiyoji Hirata**
(大友 喜佐雄) (平田 千代二)

Aus der Med. Klinik von Prof. Dr. T. Kurokawa, Tohoku Universität, Sendai

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Nach der Entdeckung des Salvarsans von Ehrlich[1] sind zahlreiche chemotherapeutische Mittel von verschiedenen Forschern wie Domagk,[2] Fleming,[3] Waksman usw.[4] hergestellt worden. Aber die Meinungen der Autoren gehen auseinander hinsichtlich der Bedeutung des RES bei dem Wirkungsmechanismus der Chemotherapeutika.

Deshalb haben wir in der vorliegenden Arbeit untersucht, welchen Einfluss Chemotherapeutika, besonders Sulfonamide und Antibiotika auf die Funktion des RES und der Leber ausüben. Die Auswahl der Versuchstiere sowie die Methodik war die gleiche wie bei der früheren Mitteilung.[5]

Versuchsergebnisse

1. Einfluss verschiedener Sulfonamide

Wie aus Tabelle I zu sehen ist, ruft die intravenöse Injektion von Aktiweis (Sulfanilamide), Trianon (Sulfapyridine), Neogerison (Acetosulfamine), Typhogerison (Acetosulfamine mit Bakterienfiltrat) oder Domian (Sulfapyrimidine), in Menge von 100 mg pro kg, eine Verminderung des Kongorotindex und gleichzeitig eine leichte Vermehrung der Prothrombinzahl hervor. In ähnlicher Weise vermindert sich der Kongorotindex bei intravenöser Injektion von Romezin (Sulfamerazine), Sulzol (Sulfathiazol), Sulzo-S (Sulfaminothiazol), Neodiseptal (Sulfamethylthiazol), Protomin (Promin) oder Thiasin (Sulfaisoxazol), in Menge von 100 mg pro kg, dagegen bleibt die Prothrombinzahl fast unverändert. Aber die Injektion von Theradiazine (Sulfadiazine) im Menge von 100 mg

TABELLE I

Einfluss verschiedener Sulfonamide

	Dose (pro kg)		Nr. des Kaninchens	Vor Inj.	Zeit nach Injektion (St.) 1	3	5
Aktiweis	100 mg i.v.	K-index	1	34.3	40.2	49.0	38.4
			2	33.5	39.1	47.2	46.6
			Durchschn.	33.9	39.6	46.9	42.6
			Differenz		-8.1	0.3	-1.5
		P-zahl	1	1.00	1.11	1.10	1.18
			2	1.00	1.00	1.02	1.09
			Durchschn.	1.00	1.06	1.06	1.14
			Differenz		0.06	0.07	0.12
Trianon	100 mg i.v.	K-index	1	52.2	51.5	65.7	58.0
			2	47.8	52.1	53.8	57.7
			Durchschn.	49.8	54.8	59.4	57.8
			Differenz		0.5	-1.4	-8.5
		P-zahl	1	1.00	1.06	1.03	1.09
			2	1.00	1.08	1.08	1.04
			Durchschn.	1.00	1.07	1.06	1.07
			Differenz		0.06	0.07	0.04
Theradiazine	100 mg i.v.	K-index	1	57.2	67.6	59.5	66.6
			2	55.4	62.1	62.4	70.0
			Durchschn.	56.2	64.5	61.0	68.3
			Differenz		3.8	-5.2	-4.5
		P-zahl	1	1.00	0.95	1.05	0.96
			2	1.00	0.95	1.01	0.97
			Durchschn.	1.00	0.95	1.03	0.97
			Differenz		-0.06	0.01	-0.07
Romezin	100 mg i.v.	K-index	1	54.5	48.2	66.7	66.8
			2	47.1	54.7	63.2	48.4
			Durchschn.	54.2	53.7	66.0	52.8
			Differenz		-5.0	0.8	-17.9
		P-zahl	1	1.00	1.04	1.03	1.11
			2	1.00	0.99	1.05	1.07
			Durchschn.	1.00	1.02	1.04	1.09
			Differenz		0.01	0.02	0.05
Sulzol	100 mg i.v.	K-index	1	45.1	47.9	54.1	50.8
			2	35.6	49.3	37.5	42.5
			Durchschn.	40.0	48.6	45.7	45.9
			Differenz		-5.2	-7.0	-4.3
		P-zahl	1	1.00	1.05	1.01	1.06
			2	1.00	1.00	1.03	1.03
			Durchschn.	1.00	1.03	1.02	1.05
			Differenz		0.03	0.03	0.03
Neodiseptal	100 mg i.v.	K-index	1	55.6	57.5	58.5	55.2
			2	52.2	57.5	65.7	58.0
			Durchschn.	44.7	46.3	50.8	44.8
			Differenz		-2.9	-4.9	-16.6
		P-zahl	1	1.00	1.01	1.03	1.11
			2	1.00	1.09	1.07	1.04
			Durchschn.	1.00	1.05	1.05	1.08
			Differenz		0.04	0.03	0.04

Sulzol-S	100 mg i.v.	K-index	1	61.0	54.8	54.5	60.0
			2	50.0	34.5	42.8	42.8
			Durchschn.	55.9	44.8	49.5	52.8
			Differenz		-14.3	-10.7	-6.8
		P-zahl	1	1.00	1.03	1.04	1.04
			2	1.00	1.03	1.00	1.02
			Durchschn.	1.00	1.03	1.02	1.03
			Differenz		0.02	-0.01	0
Neogerison	100 mg i.v.	K-index	1	58.2	54.0	72.4	69.5
			2	39.4	44.3	54.8	43.3
			Durchschn.	49.1	49.3	64.7	56.0
			Differenz		-11.6	-3.5	-10.6
		P-zahl	1	1.00	1.01	1.03	1.11
			2	1.00	1.09	1.07	1.04
			Durchschn.	1.00	1.05	1.05	1.08
			Differenz		0.06	0.08	0.10
Typhogerison	100 mg i.v.	K-index	1	37.6	37.6	47.7	52.9
			2	36.0	43.0	51.3	56.7
			Durchschn.	37.1	40.3	49.3	55.4
			Differenz		-10.6	-0.5	8.1
		P-zahl	1	1.00	1.11	1.16	1.18
			2	1.00	1.08	1.08	1.07
			Durchschn.	1.00	1.10	1.12	1.13
			Differenz		0.10	0.09	0.11
Protomin	100 mg i.v.	K-index	1	59.2	56.0	59.2	60.0
			2	35.0	33.4	40.9	42.1
			Durchschn.	48.2	46.8	56.7	52.3
			Differenz		-15.2	-4.2	-6.1
		P-zahl	1	1.00	1.07	1.02	0.98
			2	1.00	1.01	0.97	1.06
			Durchschn.	1.00	1.04	1.00	1.02
			Differenz		0.04	0.01	0
Homosulfamin	100 mg i.v.	K-index	1	50.2	54.8	63.0	69.6
			2	31.0	39.9	46.2	51.0
			Durchschn.	40.6	47.7	56.2	62.6
			Differenz		2.6	4.6	5.5
		P-zahl	1	1.00	1.14	1.08	1.07
			2	1.00	1.12	1.11	1.01
			Durchschn.	1.00	1.13	1.10	1.04
			Differenz		0.12	0.10	0
Thiasin	100 mg i.v.	K-index	1	73.8	67.8	60.8	72.8
			2	71.7	64.8	73.7	76.7
			Durchschn.	72.8	66.7	66.2	74.4
			Differenz		-17.9	-25.7	-15.9
		P-zahl	1	1.00	0.98	0.99	1.00
			2	1.00	0.98	0.98	1.00
			Durchschn.	1.00	0.98	0.99	1.00
			Differenz		-0.01	0.02	0.02
Domian	100 mg i.v.	K-index	1	52.8	42.6	61.9	50.0
			2	49.5	48.2	63.1	60.3
			Durchschn.	51.1	45.5	62.5	54.3
			Differenz		-17.4	-7.7	-14.3
		P-zahl	1	1.00	1.07	1.07	1.01
			2	1.00	1.06	1.05	1.05
			Durchschn.	1.00	1.07	1.06	1.03
			Differenz		0.08	0.09	0.05

pro kg setzt den Kongorotindex sowie die Prothrombinzahl herab, im Gegensatz dazu ruft die Injektion von Homosulfamin (Marfanil) eine Vermehrung des Kongorotindex und der Prothrombinzahl hervor.

Daraus ist ersichtlich, dass diese Sulfonamide, nicht aber Homosulfamin, die Funktion des RES steigern, dagegen wird diejenige der Leberzellen nur durch Aktiweis, Trianon, Neogerison, Typhogerison, Domian und Homosulfamin erhöht.

2. Einfluss der Antibiotika

Wenn man Penicillin G, Mix oder K, in Menge von 20000 EH intramuskulär injiziert, so vermindert sich, wie Tabelle II zeigt, der Kongorotindex und die Prothrombinzahl vermehrt sich in leichtem Grade. Aber der Kongorotindex vermindert sich und die Prothrombinzahl bleibt unverändert durch wässeriges Penicillin G in Menge von 30000 EH, während der Kongorotindex bei intramuskulärer Injektion des Penicillins K in Menge von 10000 EH oder des öligen Penicillins G in Menge von 30000 EH unverändert bleibt.

Aus Tabelle III sieht man, dass die Injektion von Streptomycin in Menge von 20000 EH, ja sogar 5000 EH den Kongorotindex herabsetzt, doch erhöht sie die Prothrombinzahl. Aber die Injektion von Colistin in Menge von 100000 EH verursacht eine Verminderung des Kongorotindex sowie der Prothrombinzahl.

Deshalb ist es klar, dass diese Antibiotika, nicht aber Penicillin in kleiner Menge und öligem Penicillin, die Funktion des RES ziemlich auffallend, aber diejenige der Leberzellen nur leichtgradig steigern.

3. Einfluss der Tuberkulostatika

Wie schon erwähnt, steigert das als Tuberkulostatika ausgezeichnete Streptomycin und Protomin die Funktion des RES. Tabelle IV zeigt, dass die subkutane Injektion von PAS (Paraaminosalicylsäure) in Menge von 200 mg pro kg den Kongorotindex erhöht, aber in Menge von 33 mg pro kg denselben herabsetzt, während er durch PAS in Menge von 50 mg pro kg fast unverändert bleibt. Weiterhin bemerken wir, dass die subkutane Injektion von Tibione (Thiosemicarbazone) in Menge von 10 mg pro kg den Kongorotindex auffällig herabsetzt, während derselbe durch Tibione in Menge von 50 mg pro kg fast unbeeinflusst bleibt. Aber die Prothrombinzahl verändert sich dabei keinesfalls. Wenn man Cepharantin, das von Hasegawa[6] als Tuberkulostatika empfohlene Mittel, in Menge von 0.05 mg pro kg injiziert, vermindert sich der Kongorotindex und vermehrt sich die Prothrombinzahl in mässigem Grade.

Aus den obigen Versuchen wird klar, dass diese antituberkulösen Mittle in geeigneter Menge die Funktion des RES steigern, aber nicht die Leberzellenfunktion, mit Ausnahme von Cepharantin.

4. Einfluss der antisyphilitischen Mittel

TABELLE II

Einfluss des Penicillins

	Dose (pro kg)		Nr. des Kaninchens	Vor Inj.	Zeit nach Injektion (St.)		
					1	3	5
Penicillin G	20000 EH i.m.	K-index	1	54.1	55.4	59.2	61.8
			2	53.6	57.2	56.3	53.1
			Durchschn.	53.9	56.2	57.9	59.0
			Differenz		-11.5	-8.7	-5.1
		P-zahl	1	1.00	1.03	1.11	1.16
			2	1.00	1.02	1.03	1.09
			Durchschn.	1.00	1.03	1.07	1.13
			Differenz		0.03	0.08	0.11
Penicillin Mix	20000 EH i.m.	K-index	1	45.9	43.3	51.5	54.6
			2	43.9	48.0	56.0	51.5
			Durchschn.	44.9	45.4	53.6	52.9
			Differenz		-13.3	-3.4	-2.2
		P-zahl	1	1.00	1.10	1.04	1.08
			2	1.00	1.02	1.09	1.02
			Durchschn.	1.00	1.06	1.07	1.05
			Differenz		0.06	0.08	0.03
Penicillin K	20000 EH i.m.	K-index	1	42.7	45.0	48.0	66.7
			2	28.9	33.1	39.0	44.1
			Durchschn.	36.7	39.9	44.0	57.2
			Differenz		-10.6	-5.4	10.3
		P-zahl	1	1.00	1.01	1.06	0.99
			2	1.00	1.02	1.04	0.99
			Durchschn.	1.00	1.02	1.04	0.99
			Differenz		0.02	0.05	-0.03
	10000 EH i.m.	K-index	1	52.6	60.0	62.3	70.6
			2	45.1	51.5	53.0	67.6
			Durchschn.	48.8	55.8	58.0	69.3
			Differenz		2.5	-1.8	4.0
		P-zahl	1	1.00	1.08	1.08	1.18
			2	1.00	1.00	1.13	1.02
			Durchscun.	1.00	1.04	1.11	1.10
			Differenz		0.03	0.09	0.06
Oeliges Penicillin G	30000 EH i.m.	K-index	1	48.7	67.8	70.8	70.0
			2	48.7	57.2	56.5	64.1
			Durchschn.	48.7	62.6	63.7	67.1
			Differenz		0.1	2.3	8.2
		P-zahl	1	1.00	1.03	1.16	1.14
			2	1.00	1.03	1.02	1.03
			Durchschn.	1.00	1.03	1.09	1·09
			Diferenz		0.03	0.10	0.07
Wassriges Penicillin G	30000 EH i.m.	K-index	1	40.5	40.8	30.7	34.5
			2	26.7	24.7	25.8	30.4
			Durchschn.	34.0	33.5	28.1	32.5
			Differenz		-14.3	-18.6	-11.7
		P-zahl	1	1.00	1.01	1.04	1.00
			2	1·00	1.00	1.02	1.00
			Durchschn.	1.00	1.01	1.03	1.00
			Differenz		0.01	0.04	-0.02

Wie aus Tabelle V zu ersehen ist, erhöht die intravenöse Injektion von Mapharsol (Mapharsene), den antisyphilitischen Arsenpräparaten, in Menge von 5 mg pro kg den Kongorotindex, dagegen setzt dieselbe Injektion in Menge von 0.5 mg pro kg den Kongorotindex herab, indem

TABELLE III

Einfluss anderer Antibiotika

	Dose (pro kg)		Nr. des Kaninchens	Vor Inj.	Zeit nach Injektion (St.)		
					1	3	5
Streptomycin	20000 EH i.m.	K-index	1	42.6	45.5	33.9	47.7
			2	41.9	55.2	52.7	57.0
			Durchschn.	42.3	50.0	45.3	52.3
			Differenz		-6.1	-9.4	-0.2
		P-zahl	1	1.00	1.01	1.05	1.09
			2	1.00	1.04	1.07	1.06
			Durchschn.	1.00	1.03	1.06	1.08
			Differenz		0.03	0.07	0.06
	5000 EH i.m.	K-index	1	47.0	53.2	57.5	52.2
			2	41.6	45.3	56.0	53.2
			Durchschn.	44.3	49.6	56.8	52.5
			Differenz		-8.5	-0.2	-2.4
		P-zahl	1	1.00	1.03	1.10	1.05
			2	1.00	0.97	1.07	1.06
			Durchschn.	1.00	1.00	1.09	1.06
			Differenz		0	0.10	0.04
Colistin	100000 EH i.m.	K-index	1	50.0	45.3	47.8	58.8
			2	25.0	30.5	35.1	50.0
			Durchschn.	37.5	38.1	41.6	54.3
			Differenz		-13.2	-8.6	6.6
		P-zahl	1	1.00	1.03	0.95	1.05
			2	1.00	1.01	1.02	1.01
			Durchschn.	1.00	1.02	0.99	1.03
			Differenz		0.02	0	0.01

sie die Prothrombinzahl erhöht. Wenn man aber Neoneonatvarsan (Neosalvarsan) in Maenge von 0.5 mg pro kg intravenös injiziert, so ist eine Verminderung des Kongorotindex sehr gering, auch bleibt die Prothrombinzahl fast unverändert.

Daraus wird klar, dass diese Arsenpräparate in kleiner Menge die Funktion des RES steigern.

5. Einfluss des Luminals auf die Wirkung der Sulfonamide und Antibiotika

Aus Tabelle VI ersieht man, dass die oben erwähnte Verminderung des Kongorotindex nach der Injektion von Neogerison, Penicillin K oder Streptomycin durch Luminal fast völlig gehemmt wird, wobei eine Veränderung der Prothrombinzahl nicht einheitlich ist.

Also ist zu vermuten, dass diese Sulfonamide und Antibiotika vermittelst des vegetativen Nervenzentrums in ähnlicher Weise wie bei Vitamin die Funktion des RES stimulieren.

6. Einfluss der Antibiotika auf die Phagozytose der Leukozyten

TABELLE IV

Einfluss der Tuberkulostatika

	Dose (pro kg)		Nr. des Kaninchens	Vor Inj.	Zeit nach Injektion (St.)		
					1	3	5
PAS	200 mg s.c.	K-index	1	53.5	80.8	62.8	61.3
			2	44.7	57.3	66.7	76.0
			Durchschn.	48.6	68.2	64.9	68.4
			Differenz		16.4	12.0	16.1
		P-zahl	1	1.00	1.02	1.04	1.02
			2	1.00	0.99	1.01	1.01
			Durchschn.	1.00	1.01	1.03	1.02
			Differenz		0	0	-0.01
	50 mg s.c.	K-index	1	44.1	44.7	46.5	44.3
			2	34.6	43.2	45.8	44.1
			Durchschn.	39.3	43.9	45.6	44.3
			Differenz		0.2	2.0	1.3
		P-zahl	1	1.00	0.98	0.93	1.03
			2	1.00	1.00	1.00	1.00
			Durchschn.	1.00	0.99	0.97	1.02
			Differenz		-0.02	-0.06	-0.01
	33 mg s.c.	K-index	1	65.7	43.9	65.7	71.8
			2	61.1	40.8	48.3	70.0
			Durchschn.	63.6	42.2	57.1	71.1
			Differenz		-24.6	-10.8	3.8
		P-zahl	1	1.00	1.01	1.02	1.04
			2	1.00	1.00	1.00	1.02
			Durchschn.	1.00	1.01	1.01	1.03
			Differenz		0	-0.02	0
Tibione	50 mg s.c.	K-index	1	48.0	74.0	77.3	63.5
			2	42.9	53.2	63.2	51.8
			Durchschn.	45.6	63.7	70.2	57.7
			Differenz		7.1	5.5	-5.4
		P-zahl	1	1.00	0.96	0.91	0.91
			2	1.00	0.92	0.89	0.89
			Durchschn.	1.00	0.94	0.90	0.90
			Differenz		-0.05	-0.07	-0.08
	10 mg s.c.	K-index	1	61.3	44.7	60.8	65.3
			2	46.8	44.8	51.1	56.3
			Durchschn.	54.3	42.7	54.0	61.2
			Differenz		-13.4	-19.4	-10.6
		P-zahl	1	1.00	0.97	1.05	1.00
			2	1.00	1.04	0.98	1.00
			Durchschn.	1.00	1.01	1.02	1.00
			Differenz		0	0.05	0.02
Cepharantin	0.05 mg s.c.	K-index	1	60.0	73.6	77.8	67.9
			2	43.1	53.9	77.5	66.7
			Durchschn.	50.3	56.7	77.8	66.8
			Differenz		-5.4	8.4	-1.0
		P-zahl	1	1.00	1.17	1.10	1.04
			2	1.00	1.08	1.02	1.02
			Durchschn.	1.00	1.13	1.06	1.03
			Differenz		0.14	0.09	0.05

Wenn man die Phagozytose der Leukozyten nach Sugiyama[7] bestimmt, so sieht man aus Tabelle VII, dass die Phagozytose der Leukozyten durch Penicillin Mix, K, Streptomycin oder Mapharsol in kleiner Menge auffallend gesteigert wird, während diese Wirkung durch Luminal

TABELLE V

Einfluss der Antisyphilitika

	Dose (pro kg)		Nr. des Kaninchens	Vor Inj.	Zeit nach Injektion (St.)		
					1	3	5
Mapharsene	5 mg i.v.	K-index	1	45.9	50.0	68.2	64.0
			2	41.5	48.7	54.5	58.3
			Durchschn.	43.5	49.3	61.1	61.2
			Differenz		-8.0	4.9	7.5
		P-zahl	1	1.00	1.01	1.05	1.02
			2	1.00	1.00	1.01	1.00
			Durchschn.	1.00	1.00	1.03	1.01
			Differenz		0	0.04	-0.01
	0.5 mg i.v.	K-index	1	38.3	37.3	38.9	42.6
			2	36.8	32.9	33.8	45.9
			Durchschn.	37.5	34.6	36.5	44.3
			Differenz		-16.7	-13.7	-5.4
		P-zahl	1	1.00	1.00	1.01	1.10
			2	1.00	1.02	1.05	1.09
			Durchschn.	1.00	1.01	1.03	1.10
			Differenz		0.01	0.04	0.08
Neosalvarsan	0.5 mg i.v.	K-index	1	45.3	51.7	58.8	63.1
			2	41.2	58.8	50.0	55.2
			Durchschn.	43.1	55.3	54.2	58.8
			Differenz		-1.6	-1.6	4.5
		P-zahl	1	1.00	0.97	0.98	1.02
			2	1.00	1.00	0.98	1.01
			Durchschn.	1.00	0.99	0.98	1.02
			Differenz		-0.01	-0.01	0

wieder völlig gehemmt wird.

Daraus wird klar, dass diese Antibiotika nicht nur die Funktion des RES, sondern auch diejenige der Leukozyten steigern.

DISKUSSION

Die Bedeutung des RES beim Wirkungsmechanismus der Chemotherapeutika ist bis heute von verschiedenen Autoren bestritten worden: einerseits berichten Kritschewsky,[8] Jungeblut[9] und Feld[10] über eine dyschemotherapeutische Wirkung bei der Milzexstirpation und der reticuloendothelialen Blockade, anderseits betonten Kikuth[11] und Prigge[12], dass dem sogenannten Reticuloendothel keine besondere Bedeutung für die Wirkung der Chemotherapeutika beigemessen werden kann. Weiterhin bemerkte Jansco,[13] dass die Wirkung der Chemotherapeutika sich mit der biologischen Schutzfunktion des Reticuloendothels kombiniert.

In Bezug auf den Einfluss der Sulfonamide berichtet Oh[14] über die Unbeeinflussbarkeit, während Morioka[15] eine Vermehrung des Kon-

TABELLE VI

Einfluss des Luminals auf die Wirkung der Chemotherapeutika

	Dose (pro kg)		Nr. des Kaninchens	Vor Inj.	Zeit nach Injektion (St.)		
					1	3	5
Luminal	30 mg i.m.	K-index	1	50.0	55.6	68.0	65.1
			2	45.8	70.6	66.7	65.0
			Durchschn.	67.8	63.0	67.3	65.5
			Differenz		13.0	15.2	14.0
		P-zahl	1	1.00	1.00	1.00	0.96
			2	1.00	1.00	0.99	0.92
			Durchschn.	1.00	1.00	1.00	0.94
			Differenz		0	0.01	-0.07
Neogerison	100 mg i.v.	K-index	1	58.2	54.4	72.4	69.5
			2	39.4	44.3	54.8	43.3
			Durchschn.	49.1	49.3	64.7	56.0
			Differenz		-11.6	-3.5	-10.6
		P-zahl	1	1.00	1.01	1.03	1.11
			2	1.00	1.09	1.07	1.04
			Durchschn.	1.00	1.05	1.05	1.08
			Differenz		0.06	0.08	0.10
Neogerison nach Luminal	Neogerison 100 mg i.v. Luminal 30 mg i.m.	K-index	1	32.3	54.4	72.2	70.2
			2	26.1	41.1	58.5	65.0
			Durchschn.	29.5	48.8	65.8	67.3
			Differenz		5.5	23.6	27.2
		P-zahl	1	1.00	1.02	1.04	1.02
			2	1.00	0.98	0.95	0.92
			Durchschn.	1.00	1.00	1.00	1.02
			Differenz		0	0.01	-0.05
Penicillin K	20000 EH i.m.	K-index	1	42.7	45.0	48.0	66.7
			2	28.9	33.1	39.0	44.1
			Durchschn.	36.7	39.9	44.0	57.2
			Differenz		-10.6	-5.4	10.3
		P-zahl	1	1.00	1.01	1.06	0.99
			2	1.00	1.03	1.02	0.99
			Durchschn.	1.00	1.02	1.04	0.99
			Differenz		0.02	0.05	-0.03
Penicillin K nach Luminal	Penicillin K 20000 EH i.m. Luminal 30 mg. i.m.	K-index	1	43.0	75.8	72.1	70.0
			2	39.1	51.2	71.3	68.0
			Durchschn.	41.1	64.1	71.8	69.1
			Differenz		18.5	19.7	10.5
		P-zahl	1	1.00	1.00	1.13	1.13
			2	1.00	0.91	0.99	1.03
			Durchschn.	1.00	0.96	1.06	1.08
			Differenz		-0.05	0.04	0.04
Streptomycin	20000 EH i.m.	K-index	1	42.6	45.4	33.9	47.7
			2	41.9	55.2	52·7	57.0
			Durchschn.	42.3	50.0	45.3	52.3
			Differenz		-6.1	-9.4	-0.2
		P-zahl	1	1.00	1.01	1.05	1.09
			2	1.00	1.04	1.07	1.06
			Durchschn.	1.00	1.03	1.06	1.08
			Differenz		0.03	0.07	0.06
Streptomycin nach Luminal	Streptomycin 20000 EH i.m. Luminal 30 mg i.m.	K-index	1	37.3	48.0	62.0	80.0
			2	34.4	50.0	60.8	63.1
			Durchschn.	35.7	49.1	61.2	71.5
			Differenz		-0.4	12.8	25.6
		P-zahl	1	1.00	1.02	1.08	1.05
			2	1.00	0.99	1.03	1.04
			Durchschn.	1.00	1.01	1.06	1.05
			Differenz		0.01	0.07	0.03

TABELLE VII

Einfluss der Chemotherapeutika auf die Phagozytose der Leukozyten

	Dose (pro kg)		Vor Inj.	Zeit nach Injektion (St.)					
				1	2	3	4	5	6
Kontrolle		Phagozytose	1.46	1.41	1.23	1.25	1.19	1.40	1.42
		Differenz		-0.05	-0.23	-0.21	-0.27	-0.06	-0.04
Penicillin Mix	20000 EH i.m.	Phagozytose	1.90	1.77		2.07		1.95	
		Differenz		-0.06		0.40		0.13	
Penicillin K	20000 EH i.m.	Phagyzytose	1.27	1.49	0.69	1.25	1.23	1.14	1.15
		Diflerenz		0.27	-0.35	0.19	0.23	-0.07	-0.08
Streptomycin	20000 EH i.m.	Phagozytose	1.97	1.93	2.03	1.74	1.99	2.04	2.00
		Differenz		0.03	0.31	0	0.31	0.15	0.03
Mapharsol	5 mg i.m.	Phagozytose	2.20	1.63	1.11		0.77	0.52	0.71
		Differenz		-0.52	-0.86		-1.16	-1.62	-1.45
	0.5 mg i.v.	Phagozytose	1.11	1.26	1.20	0.94	0.78	0.43	0.33
		Differenz		0.20	0.32	0.04	-0.06	-0.62	-0.74
Streptomycin nach Luminal	Streptomycin 20000 EH i.m. Luminal 30 mg i.m.	Phagozytose	1.66	1.52	1.07		1.12		1.25
		Differenz		-0.04	-0.36		-0.27		0.37

gorotindex bemerkt und Mizuta[16] eine Steigerung der Farbstoffausscheidungsfunktion der Leber beobachtet. Weiterhin betonen Finkelstein,[16] Neufeld[18] und Tomizuka[19] eine Steigerung der Phagozytose der Leukozyten durch Sulfonamide, doch negieren Sandkühler,[20] Kubo usw.[21] diese Wirkung.

Hinsichtlich der Wirkung der Antibiotika berichtet Sakata,[22] dass Streptomycin die Funktion des RES bei Tuberkulosekranken steigert. Weiterhin betonen Tomizuka,[19] Kimura[23] und Harakawa[24] eine Vermehrung der Phagozytose sowie der Wanderungsgeschwindigkeit der Leukozyten durch Streptomycin. Vor allem bemerken Kubo usw.[21] dass nicht nur Streptomycin, sondern auch Penicillin die Phagozytose der Leukozyten steigern, während Sandkühler[20] eine solche Wirkung von Penicillin negiert.

Betreffs der antisyphilitischen Arsenpräparate bemerken Goldzieher und Peck[25] eine Steigerung der Funktion des RES, aber Haendel und Malet[26] eine Herabsetzung derselben. Weiterhin betont Iwashita[27] eine Verminderung der Funktion des RES bei grosser Dose und eine Vermehrung derselben bei kleiner Dose.

Aus unseren Versuchsergebnissen ist ersichtlich, dass verschiedene Sulfonamide und Antibiotika, mit Einschluss von antituberkulösn sowie antisyphilitischen Mitteln, die Funktion des RES sowie die Phagozytose der Leukozyten steigern, dagegen ist ihr Einfluss auf die Leberzellenfunktion

nicht einheitlich. Sogar diese Wirkung der Sulfonamide und Antibiotika wird durch Luminal ganz aufgehoben. Deshalb ist zu vermuten, dass diese Sulfonamide und Antibiotika vermittelst des vegetativen Nervenzentrums, in ähnlicher Weise wie bei Vitamin, die Funktion des RES stimulieren.

Infolgedessen ist es nicht zu leugnen, dass sich die Funktion des RES beim Wirkungsmechanismus der Sulfonamide sowie der Antibiotika, mit Einschluss von antituberkulösen Mitteln, beteiligt.

Zusammenfassung

Wenn wir unsere Versuchsergebnisse zusammenfassen, so können wir folgenderweise schliessen:

1. Von den verschiedenen Sulfonamiden rufen Aktiweis, Trianon Theradiazine, Romezin, Sulzol, Sulzol-s, Neodiseptal Neogerison, Typhogerison, Protomin, Thiasin und Domian, nicht aber Homosulfamin, eine Verminderung des Kongorotindex hervor, dagegen steigt die Prothrombinzahl nur durch Aktiweis, Trianon, Neogerison, Typhogerison, Homosulfamin und Domian.

2. Von den verschiedenen Antibiotika setzen Penicillin G, Mix, K, wässriges Penicillin G, Streptomycin und Colistin, nicht aber Penicillin K in kleiner Menge und öligem Penicillin G, den Kongorotindex herab. Dabei wird die Prothrombinzahl durch Penicillin G, Mix, K und Streptomycin gesteigert, sonst aber bleibt sie unverändert.

3. Verschiedene Tuberkulostatika wie PAS, Tibione und Cepharantin setzen den Kongorotindex nur bei geeigneter Menge herab, aber die Prothrombinzahl bleibt dabei fast unverändert, mit Ausnahme von Cepharantin.

4. Mapharsol, das antisyphilitische Arsenpräparat, setzt den Kongorotindex nur bei kleiner Dose herab und erhöht ihn bei grosser Dose, während Neoneonatvarsan auch in kleiner Menge eine sehr geringe Verminderung des Kongorotindex verursacht.

5. Die oben erwähnte Herabsetzung des Kongorotindex bei Neogerison, Penicillin K und Streptomycin wird durch Luminal völlig gehemmt.

6. Verschiedene Antibiotika wie Penicillin Mix, K und Streptomycin steigern die Phagozytose der Leukozyten, aber diese Wirkung wird durch Luminal wieder völlig gehemmt.

7. Daraus ist ersichtlich, dass diese Sulfonamide und Antibiotika die Funktion des RES und der Leukozyten vermittelst des vegetativen Nervenzentrums steigern.

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A Mucopolysaccharide from Human Cerebral Cortex*

By

Tatsunori Ejima
(江島 達憲)

From the Medico-chemical Institute (Director: Prof. H. Masamune) and the Department of Neuropsychiatry (Director: Prof. T. Ishibashi), Tohoku University, Sendai

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A mucopolysaccharide was isolated from human cerebral cortex and its several properties were examined.

Experimental

Preparation Procedure

Material. Cerebri from 6 humans all belonging to Blood Group O were freed from adhesive membranes and blood vessels mechanically as far as possible and divided into cortex and medulla, and the cortices were minced. 2772 g. of a brei thus obtained were covered under 4.5 volumes of 91% denatured alcohol containing about 3% methanol for one week, then squeezed and dried in a Faust apparatus. The material dried weighed 392 g. It was further defatted by boiling with 5 and 3.5 volumes of abs. alcohol for 1 hour and 40 minutes respectively in succession and after drying in a vacuum desiccator, pulverized in a mortar. 260 g. of a brownish powder were given.

Extraction and separation of the mucopolysaccharide. The powder was shaken with 10, 5, 5 and 3 volumes of 10% NaCl for 3, 3, 4 and 4 days respectively in sequence, employing toluene as a preservative. The final solution was almost Molisch- and biuret-negative. The solutions were treated separately in similar manners. Hence the run with the first solution will be described in detail below. It was acidified with 10% HCl to pH 1.5, and the flocculent precipitate[1] was centrifuged off. The supernatant, yellowish and opalescent, was dialyzed for 3 days (preservative toluene) until the pH was raised to 6.4, and distilled in vacuo to about 20 cc. Some deposit[2], which had occurred during the dialysis, was re-

* The 173rd report of Masamune and coworkers' "Biochemical Studies on Carbohydrates."

jected and the clear fluid was added to with 5 volumes of alcohol. The deposit hereby was washed with alcohol and dried in vacuo over $CaCl_2$. 1.85 g. of a gray powder were yielded—Fr. Aa. Molisch and biuret ++, Bial-Mejbaum +, Neuberg-Saneyoshi ±, Dische −, a little P detected. From the second to fourth solutions were given 0.45, 0.49 and 0.07 g. respectively of products corresponding to the one above. These were united together (Fr. Ab).

The balance of Fr. Aa (1.80 g.) was taken up in 180 cc. of water. The yellowish turbid solution (pH 6.0) was at first acidified to pH 4.2 with 25% acetic acid, and after removal of the precipitate[3], the light yellow, almost clear mother fluid was added to with 10% NaOH to pH 5.6 and followingly with 20% barium acetate till no more occurrence of precipitate.[4] Then, the solution was alkalized to pH 9.0 with the sat. $Ba(OH)_2$ and the substance carried down[5] was discarded. The fluid was freed from barium with a just sufficient quantity of dilute sulfuric acid, dialyzed for 3 days against running tap water and distilled in vacuo to about 15 cc., followed by precipitation with 10 volumes of 99% alcohol and a little sodium acetate. The precipitate was washed with alcohol and dried. A grayish powder was given—Fr. Ba. 0.78 g. was the crop. Molisch ++, biuret ++, Bial-Mejbaum +, Dische −, P +. 0.43 g. of a resembling substance (Fr. Bb) was obtained from Fr. Ab and united with Fr. Ba—Fr. B.

Next, 1.1 g. of it were dissolved in 110 cc. of water (The pH of the solution was 6.8 and hence it was lowered to 5.8 with a dilute H_2SO_4) and saturated with $(NH_4)_2SO_4$. The voluminous flocculent precipitate was centrifuged off.[6] The supernatant was dialyzed for 5 days until Nessler test resulted in negative, and distilled in vacuo to about 10 cc., then the condensate was precipitated with abs. alcohol containing sodium acetate, washed with alcohol and dried. A gray powder amounting to 322 mg. was given. Molisch ++, biuret +, Bial-Mejbaum +, Dische −, P ±, Neuberg-Saneyoshi ±. Since it still showed the positive biuret reaction, it was again fractioned with $(NH_4)_2SO_4$ as above. A flocculent precipitate on saturating with the salt amounted even to 75 mg.[7] and gave strong biuret reaction[8], but the fraction recovered from the mother fluid (a white powder weighing 184 mg.)—Fr. C—was also biuret-positive and contained in addition some phosphorus and hexuronic acid (Neuberg-Saneyoshi).[9]

Finally, Fr. C was purified by the aid of lead acetate:—170 mg. of it were taken up in 15 cc. of water. As the light brown, clear solution was slightly alkaline (pH 7.2), it was acidified to pH 5.6 with dilute acetic acid, and 10% lead acetate was added in small portions. The precipitate[10] was centrifuged off and the supernatant (yellowish and transparent;

pH 5.6) was treated with H_2S and filtered with suction. The filtrate was nearly neutralized with NaOH and distilled under reduced pressure to about 5 cc., and 10 volumes of alcohol containing sodium acetate were added. The deposit obtained was washed with alcohol and dried. 126 mg. was the yield of a white powder given. Molisch ##, biuret −, Bial-Mejbaum ±, Dische −, P −, Neuberg-Saneyoshi −, direct Osaki-Turumi ±, indirect Osaki-Turumi +.

Properties of the Mucopolysaccharide

Solubility. It was clearly soluble in water of various pH's, 5% trichloroacetic acid and the saturated $(NH_4)_2SO_4$, and gave no precipitate with cupric chloride, barium acetate, baryta and lead acetate.

Electrophoresis. It showed only one single boundary after electrophoresis at pH 7.8, as is shown in Fig. 1.

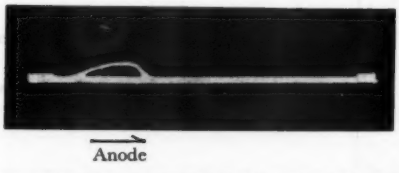

Fig. 1. Electrophoretic pattern of a 1% solution of the cerebral mucopolysaccharide in phosphate buffer, pH 7.8, I 0.29; current 10 mA; exposure 45 minutes; temperature 17.5°C. Ascending limb only is shown.

Paper partition chromatography of the sugar components. The procedure of Masamune and Yosizawa[11] was adapted. 35.2 mg. of the substance were hydrolyzed with 3 cc. of 1 *N* H_2SO_4 in an ampoule at 100°C for 4½ hours, and the hydrolysate freed from most of H_2SO_4 was distilled in vacuo to about 0.5 cc. 0.015 cc. of the condensate was sampled on paper slivers 5 cm. × 60 cm. square for the descending chromatography. Solvents, *n*-butylacetate-acetic acid-ethanol-water (3:2:1:1) and butanol-pyridine-water (5:3:2). The standard solution was 2% with respect to individual sugars and 0.01 cc. was put on the papers. In the chromatograms were shown distinct spots corresponding to chondrosamine, glucosamine and galactose and weak spots corresponding to mannose and L-fucose (Fig. 2).

Quantitative analysis. The substance was dried to a constant weight in vacuo over $CaCl_2$ for the analysis. The figures obtained are embodied in Table I.

Optical rotation in water. $[\alpha]_D^{21}=\dfrac{-0.29\times 1}{0.0091\times 1}=-31.9°$

Retention analysis. Hakomori[15] mentioned that retention analysis of

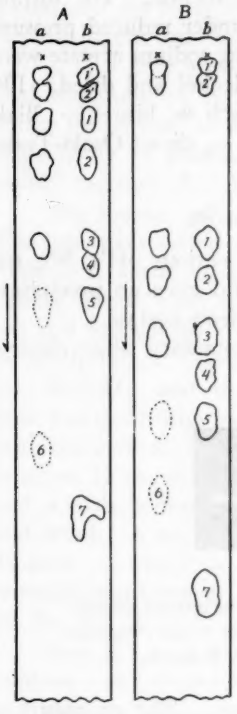

Fig. 2. Diagrammatical reproductions of the *n*-butylacetate-acetic acid-ethanol-water (3 : 2 : 1 : 1) (A) and butanol-pyridine-water (5 : 3 : 2) (B) chromatograms of the hydrolysate of the cerebral mucopolysaccharide. Temperature and duration of test 22°C and 24 hrs. respectively. Indicator aniline hydrogen phthalate of Partridge.

a: the mucopolysaccharide, b: reference run, 1, 1′: chondrosamine sulfate, 2, 2′: glucosamine sulfate, 3: galactose, 4: glucose, 5: mannose, 6: L-fucose, 7: rhamnose.

TABLE I

Composition of the Cerebral Mucopolysaccharide

Analysis	In per cent	In equivalents per equivalent weight
N*	4.8	4.9
Hexosamines as glucosamine†,	12.5	1.0
Galactose‡	32.8	2.6
Mannose‡	14.1	1.1
L-Fucose§	7.6	0.7
Ash//	8.6	

* Micro Kjeldahl, † Blix[12], ‡ Masamune and Ogawa[13], § Masamune and Ogawa[14], // Pregl without use of H_2SO_4.

Wieland & Fischer suggests N-glycoside linkage, if enlargement of the gap of the copper field due to a substance is caused by prior heating of the substance with mild acid. Hence, of two 0.17 cc. portions of a 1.25% so-

lution of the mucopolysaccharide in 1 *N* acetic acid, one was maintained at 20°C for 5 hours and the other at 50°C for the same duration of time. They were followingly distilled at 20°C to dryness and the still-residues were taken up each in 0.085 cc. of water. Then, two 15 cm. × 10 cm. squares of the Toyo Roshi filter paper No. 2 were each spotted with a 0.03 cc. portion of a 2.5% solution in water of the substance as such and one or the other of the solutions above side by side at equal distances from the long-side bottom, and subjected to the procedure described by Hakomori. Temperature of irrigation 20°C. As is reproduced in Fig. 3, the gap of the copper field due to the substance, which had been heated at 50°C with the acid, was markedly larger than that due to the substance untreated, while no noticeable enlargement of gap was recognized to have been caused by

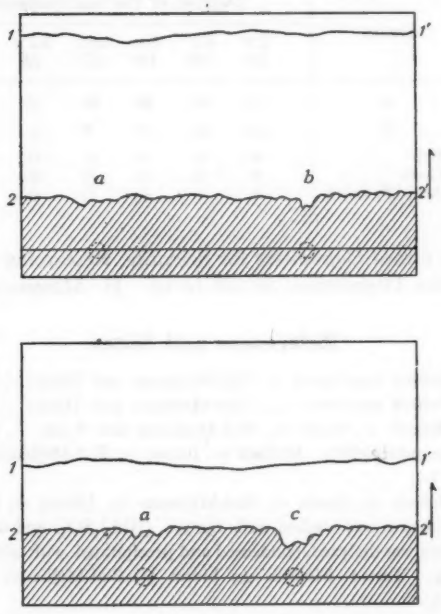

Fig. 3. Retention analysis according to Wieland & Fischer. a: the mucopolysaccharide as such, b: the mucoplysaccharide maintained at 20°C in contact with the acetic acid. c: the mucoplysaccharide heated with the acetic acid at 50°C.
1—1': tetrahydrofurane front, 2—2': copper front.

the substance stood at 20°C with the acid. Judging from the results, N-glycoside link is involved most probably in make up of the mucopolysaccharide.

Group specificity. The substance was examined regarding group potency by the method detailed in the article of Masamune *et al.*[16] As shown in Table II, it proved non-group active.

TABLE II

Occurrence of Agglutination of A, B and O Erythrocytes by Respective Antisera, treated with the Cerebral Mucopolysaccharide

The normal α, β, eel and goat sera and anti-human OS saliva chicken serum had titres 160, 640, 2560, 160 and 1280 respectively and were all diluted to titre 16. ± : faint agglutination, + : slight aggl., ++ : marked aggl., +++ : strong aggl.

Group of red cells	Serum	Dilution of the mucopolysaccharide (1 :)							
		2×10^2	4×10^2	8×10^2	1.6×10^3	3.2×10^3	6.4×10^3	1.28×10^4	∞
A	α	++	++	+++	+++	+++	+++	+++	+++
B	β	±	±	+	+	+	++	+++	+++
O	Eel-	+	+	+	++	++	+++	+++	+++
	Goat-	+	+	++	+++	+++	+++		+++
	Anti-OS saliva	+	+	+	++	++	+++		+++

Through the Grant Committee for Scientific Researches was given a grant from the Education Department in aid to us. H. Masamune.

References and Notes

1) Tests: Molisch and biuret ++, Bial-Mejbaum and Dische +, P ++.

2) Tests: Molisch and biuret ±, Bial-Mejbaum and Dische −, P +.

3) Tests: Molisch +, biuret ++, Bial-Mejbaum and P-test +, Dische −.

4) Tests of the precipitate: Molisch ++, biuret +, Bial-Mejbaum ++, Dische −, P +, Neuberg-Saneyoshi +.

5) Tests: Molisch +, biuret ++, Bial-Mejbaum ±, Dische −, P +.

6) The centrifugate was washed with the sat. $(NH_4)_2SO_4$, and then dissolved in 20 cc. of water. The solution was dialyzed, condensed and precipitated with alcohol. The substance dried weighed 0.51 g. Tests: Molisch ±, biuret +++, Bial-Mejbaum +, Dische −, P ±, Neuberg-Saneyoshi ±.

7) The substance was recovered after dialysis and dried.

8) Further tests: Molisch +, Bial-Mejbaum +, Dische −, P ±, Neuberg-Saneyoshi ±.

9) Further tests: Molisch +++, Bial-Mejbaum +, Dische −.

10) The precipitate was suspended in water and after passing of H_2S, filtered with suction. The filtrate was condensed in vacuo and precipitated with alcohol. It proved Molisch +, biuret +++, Bial-Mejbaum −, Dische −, P ± and Neuberg-Saneyoshi ±.

11) Masamune & Yosizawa, Tohoku J. Exp. Med., 1953, **59**, 1.

12) Blix, Acta chem. Scand., 1948, **2**, 467.

13) Masamune & Ogawa, Tohoku J. Exp. Med., 1954, **60**, 11.

14) Masamune & Ogawa, Tohoku J. Exp. Med., 1954, **60**, 33.
15) Hakomori, *ibid.*, 1954, **60**, 331.
16) Masamune, Yosizawa & Masukawa, *ibid.*, 1953, **58**, 381.

Note at proof-reading: This article was read before the 51st Annual Meeting of the Japanese Society for Psychiatry and Neurology, Nagoya, April 5-7, 1954.

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Chemical and Immunological Study of DNA in particular of Cancer DNA*

By

Hajime Masamune, Shigeru Tsuiki, Eiitiroh Kitabatake and
(正宗 一) (立木 蔚) (北畠 英一郎)
Mutuo Sakamoto
(阪本 睦男)

From the Medico-chemical Institute (Director: Prof. H. Masamune) and the Forensic Medical Institute (Director: Prof. T. Murakami), Tohoku University, Sendai

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The Group A mucopolysaccharide from stomach cancer is composed essentially similar to the corresponding group mucopolysaccharide of stomach mucosa, but they differ from each other in optical rotation, group activity and partial-antigen contents.[1] Their further discrepancy has been detected in cross-reactivity of their immune antibodies with them, namely, the antibody of the cancer group carbohydrate can be absorbed entirely with the mucosa substance, whereas the antibody of the latter carbohydrate only in part by the cancer substance. It was thus assumed that the surface of the determinant group of the cancer group substance is deprived of rigidity or fine specific structure.[2] The immunological study along this line has been extended to desoxypentosenuelic acids which, whether from normal or cancerous tissues, possess the same purine and pyrimidine bases in the molecule and are said not to diverge quantitative-analytically from one another among the different organs of an animal species[3] or even among different mammals.[4]

DNAs were prepared from spleen, liver and thymus of rat, pig spleen and calf thymus besides from Yoshida sarcoma. The analyses of rat-spleen and -liver and pig spleen faithfully supported the claim of Chargaff[3] that desoxynucleic acids are characteristic in their composition of the species from which they are derived. But the DNAs of thymus and particularly of Yoshida sarcoma showed definite discrepancy, though not extreme, from the DNAs of the rat tissues above. On the other hand, DNAs of rat- and calf-thymus resembled each other in the ratios adenine/guanine and thymine/cytosine although they were not coincident with each other with respect to purines/pyrimidines, suggesting some organ-specificity.

* The 5th report of Masamune and coworkers' "Nucleic Acids".

Next, the antibodies of DNAs of rat normal tissues could not be absorbed beyond a limit even with a large amount of the Yoshida sarcoma DNA from the antisera of those tissues. On the contrary, the antibody of the sarcoma DNA was absorbed perfectly from the anti-Yoshida sarcoma rabbit serum with the DNAs of normal rat tissues. The fact is in accord with those obtained when stomach cancer group mucopolysaccharide was compared in the same serological manner with the corresponding stomach substance. Furthermore, it was proved that even the DNAs of the other species of animals are able to entirely absorb the antibody of the sarcoma DNA from the rabbit serum against Yoshida sarcoma. In other words, the results gave another example demonstrating the principal serological feature of "immature structure" in high-molecular cancer components, which are made up of the same chemical components as the corresponding components of normal tissues of the animal as regards species-specific substances or as the corresponding components of the corresponding normal tissue of the animal as regards the non-species specific. To add, in the serological experiments above, the rabbit spleen- and liver-DNA behaved similar to each other on one hand, and on the other, the Yoshida sarcoma DNA showed some peculiarity as well as decreased species-specificity and thymus DNA some organ-specificity, in harmony with the analytical findings, as will be detailed below.

Experiments with Comments

Preparation of DNAs

The total operations were processed in an unheated room (0–5°C) during winter.

1) From Yoshida sarcoma. Fresh nodules on omentum majus of this sarcoma were separated from 92 cancered rats. The total weight amounted to 485 g. They were ground without delay, roughly washed with 0.05 *M* sodium citrate to remove blood, and extracted three times in succession with 4 volumes of 0.14 *M* NaCl containing 0.01 *M* sodium citrate, each time extending over 3 hours, in order to free from the soluble proteins and PNA. The residue was then shaken with 4 volumes of 1 *M* NaCl containing 0.01 *M* sodium citrate for 3 hours, and centrifuged (4000 r.p.m., 30 min.). The centrifugate was again subjected to a similar treatment. The supernatant, which was ropy, was diluted with 6 volumes of water, whereby fibrous DNP precipitated. The second solution also gave but a small precipitate. The precipitates were combined, taken up in 300 cc. of 10% NaCl and after discarding the insoluble part, poured into 1.5 volumes of abs. ethanol. 4 reprecipitations were carried out, and the crude DNA thus given was next dissolved in 100 cc. of 10% NaCl

and deproteinized with a chloroform-amylalcohol (5 :1) mixture according to Sevag again and again, until no more gel formed. The solution was then poured into 1.5 volumes of abs. ethanol. The fibrous precipitate here was dissolved in 100 cc. of water and precipitated with 3 volumes of abs. ethanol. After 3 more repetitions of this operation (The final precipitation could be effected by addition of some NaCl in addition to abs. ethanol), it was washed with 80% ethanol, abs. ethanol and ether in turn and dried in vacuo over $CaCl_2$. Yield, 0.47 g. (0.01% of the starting fresh material).

2) From rat spleen. Prepared similar to above. Yield 0.31% of the fresh tissue.

3) From rat-liver and -thymus and pig spleen. The DNAs were prepared as above with modifications following. The rat liver brei was frozen with a dry ice-acetone mixture and the preliminary extraction was made by replacing 0.4 *M* NaCl for 0.14 *M* NaCl according to Lack[5], and the rat thymus brei was treated immediately with 1 *M* NaCl without prior extraction with 0.14 *M* NaCl. In the case of pig spleen, the product obtained as in 1) was further dialyzed against changes of distilled water in a refrigerator and precipitated with 2 volumes of abs. ethanol containing some NaCl. 0.04, 0.60 and 0.04% of the respective starting tissues were the yields of the DNAs from the rat-liver and -thymus and pig spleen.

4) From calf thymus. 3.2 g. of crude DNA were given from 250 g. of the fresh tissue by the method of Hammarsten[6]. It was purified by the Sevag procedure after dissolving in 10% NaCl as above.

Properties of the DNA Preparations

The products were biuret- and Molisch-negative. Of them, those from Yoshida sarcoma and rat normal tissues were examined electrophoretically at pH 7.7. Fig. 1 shows the patterns. Analytical figures are embodied in Table I and ultraviolet maximum and minimum extinctions in Table II. In Fig. 2 are plotted the ultraviolet extinction coefficients of Yoshida sarcoma DNA. The measurement of the absorptions was made in a Hitachi EPB-U type spectrophotometer.

All the DNAs had N/P ratio of about 1.7, but regarding the ratio adenine/guanine, pig spleen DNA differed distinctly from the others. Regarding the ratio thymine/cytosine, DNA of rat spleen resembled that of liver of the same animal, DNA of rat thymus that of calf thymus, and DNA of Yoshida sarcoma that of pig spleen (The coincidence in the last pair might be accidental), the ratio, however, diverging from one pair of the DNAs to another. Namely, 1) even among the DNAs from rat

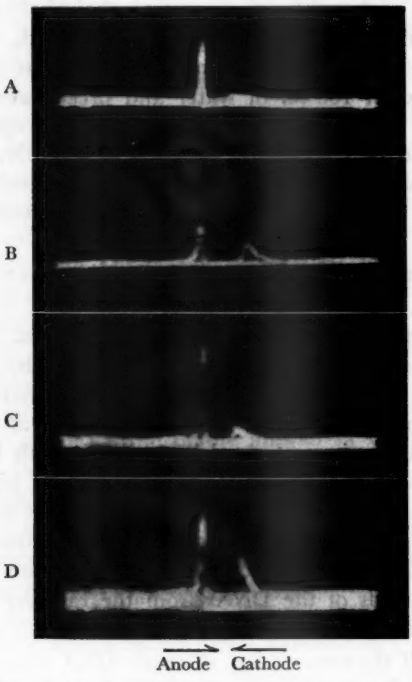

Fig. 1. Electrophoresis patterns of 0.5% solutions of DNAs of Yoshida sarcoma (A) and rat-spleen (B), -liver (C) and -thymus (D) in phosphate buffer pH 7.7, I 0.2; temp. 15°C; cur. 8 mA. Exposure after starting current 30 minutes. Mobilities:

Yoshida sarcoma DNA, $\frac{1.5\times 0.006817\times 0.3}{1800\times 0.008}=21.3$ cm^2·sec^{-1}·volt^{-1}·10^5

rat spleen DNA, $\frac{1.6\times 0.006817\times 0.3}{1800\times 0.008}=22.7$,,

rat liver DNA, ,, $=22.7$,,

rat thymus DNA, ,, $=22.7$,,

tissues, those of thymus and the sarcoma show discrepancy from those of spleen and liver, which agree mutually on their side, with respect to the contents of some of the bases, 2) the thymus DNAs of the different animals are similar with respect to thymine/cytosine to each other although their purines/pyrimidines ratios are incoincident, 3) DNA of pig spleen differs from the corresponding DNA of rat regarding any of the various ratios of the bases, revealing definite variation of DNA according to the animal species.

As to the ultraviolet extinctions, the DNAs did not agree with one

TABLE I

Composition of the DNAs from Rat-Spleen, -Liver and -Thymus, Yoshida Sarcoma, Pig Spleen and Calf Thymus

Source of DNAs	In per cent							N/P	Molecular ratio calc. to a total of 4.0				Molecular ratios			
	N*	P†	Ash‡	Adenine§	Guanine§	Cytosine§	Thymine§		Adenine	Guanine	Cytosine	Thymine	$\frac{\text{Adenine}}{\text{Guanine}}$	$\frac{\text{Thymine}}{\text{Cytosine}}$	$\frac{\text{Purines}}{\text{Pyrimidines}}$	
Rat spleen	12.6	7.3	24.5	8.4	6.8	4.4	8.4	1.72	1.17	0.82	0.77	1.24	1.43	1.61	0.99	
» liver	12.9	7.5	21.8	8.5	6.8	4.6	8.5	1.71	1.17	0.82	0.77	1.24	1.43	1.61	0.99	
» thymus	13.1	7.7	23.5	9.5	8.3	5.3	8.8	1.70	1.18	0.82	0.81	1.19	1.44	1.47	1.00	
Yoshida sarc.	13.1	7.6	22.8	9.9	6.6	5.5	8.6	1.72	1.22	0.83	0.82	1.13	1.47	1.38	1.05	
Pig spleen	13.8	8.1	20.7	10.0	6.6	5.2	8.0	1.70	1.33	0.76	0.82	1.09	1.75	1.33	1.09	
Calf thymus	12.3	7.2	24.8	8.9	6.9	4.6	7.7	1.71	1.23	0.85	0.78	1.14	1.45	1.46	1.08	

* Micro Kjeldahl, † Modified Plimmer, ‡ Pregl, § Masamune & Sakamoto[10].

TABLE II

Ultraviolet Absorption of the DNAs from Rat-Spleen, -Liver and -Thymus, Pig Spleen and Calf Thymus

Source of DNAs	Maximum Wave length (mμ)	ε(P)*	Minimum Wave length (mμ)	ε(P)*
Rat spleen	259	6580	230	2900
» liver	259	6420	230	3100
» thymus	258	6210	230	3060
Yoshida sarcoma	259	6240	230	2710
Pig spleen	260	7780	234	4540
Calf thymus	260	6880	231	2730

* Atomic extinction coefficient with respect to phosphorus, namely, 30.98 E/cl ($c=\frac{\text{g. of phosphorus}}{l. \text{ of solution}}$; l thickness of cell in cm.; $E=-\log T$)[9].

another, mostly due to different polymerization grades judging from the experience of Kunitz.[7] Besides it may be of significance that the electrophoretic mobility of Yoshida sarcoma DNA was somewhat less than the corresponding mobility of the other DNAs.

Wyatt[8] has already established the presence of a trace of 5-methylcytosine in DNAs of animals and higher plants. We could not detect it probably because of its being a tiny amount.

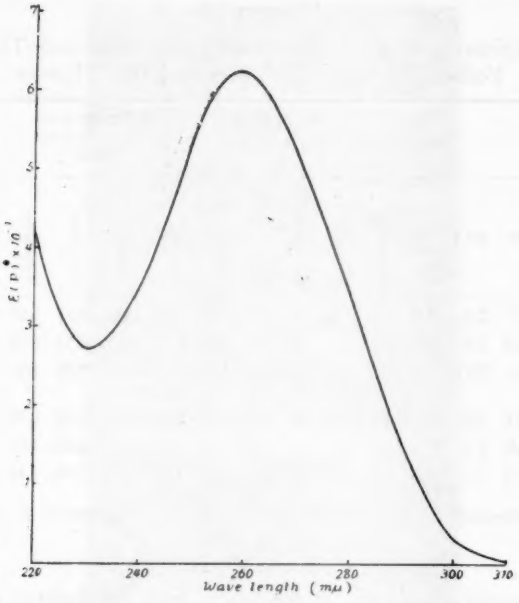

Fig. 2. Absorption spectrum of Yoshida sarcoma DNA.
* See note of Table II.

Serological Materials

Immune sera. Fresh tissue (spleen and liver of rats and Yoshida sarcoma nodules on omentum majus of rats), freed from blood by washing with ice-cooled physiological saline, was homogenized, and a part of the homogenate was diluted with 9 volumes of physiological saline for the first injection, setting aside the most part in a state freeze-dried for the following injections. A male rabbit weighing more than 2 kg. was injected for the first time with 1 cc. of the suspension above into auricular vein, and for the second and following times with 0.5 to 1 cc. of a suspension in 9 volumes of physiological saline of the preserved tissue powder subcutaneously or intraperitoneally at first and after 2 hours with 1–9 cc. intravenously in portions. The injections were made 10–12 times at intervals of 72 hours. The total volumes used of the suspensions of the tissues ranged from 60 to 80 cc. Blood was shed after 7 days from the last injection through the carotic artery, and the sera containing a sufficient amount of antibody (complement fixation test) thus obtained were inactivated at 56°C for 30 minutes and placed together with 1/10,000 volume of 1% marzonin Takeda (sodium ethylmercuric thiosalicylate) in a refrigerator.

Three samples each of anti-spleen (M_1–M_3), anti-liver (L_1–L_3) and anti-Yoshida sarcoma (Y_1–Y_3) sera were prepared. For the control runs in the experiments below, the normal sera of the rabbits later immunized were inactivated and preserved.

In vitro antigen. Fresh spleen and liver of rats and Yoshida sarcoma nodules were gound in a mortar and stirred up in 8 volumes of physiological saline separately and 5% phenol was added to 0.5%. The mixtures were shaken for 24 hours and centrifuged (3,000 r.p.m.) for 10 minutes, and the opaque supernatants were preserved. The DNAs above were also employed after dissolution in 10 volumes of the saline.

Method of Serological Assays

Complement fixation test. It was processed by employing a guinea pig serum diluted as to contain 2 complement units in 0.1 cc. and a 2.5% beef erythrocyte suspension in an immune rabbit serum, which had been diluted so that 0.25 cc. of the hemolytic system might contain 7 units hemolysin, as the complement solution and hemolytic system respectively. Regarding details, see Tokutomi.[11]

Precipitin test. The results were judged 5 hours after overlaying of a solution to be examined over the anti-serum unabsorbed or absorbed.

Absorption of immune sera. Definite amounts of DNAs (Na-salts) were separately dissolved in 1 cc. portions of the immune sera diluted twice with physiological saline, followed by standing at 37°C for 2 hours and then at 0°C overnight.

Results of the Serological Assays

1) Complement fixation test proved that in the antisera similar amounts of antibodies had been produced toward the saline extracts of the respective tissues used for immunization (Table III), but no antibodies toward DNAs of the respective tissues in so far as the results by progressive dilution of the antigens are concerned (Table IV).

2) On the contrary, the precipitin test revealed the occurrence of antibodies of the DNAs. Nevertheless, those antisera reacted not only with DNAs from the various tissues of rat, including Yoshida sarcoma, but also with those from animals belonging to different species, although the reaction intensity varied from one tissue to another (Table V).

3) Table VI shows the absorbability by the various DNAs of the DNA antibody from the serum against rat spleen. This antibody could be perfectly removed by DNAs of liver and thymus of rat, but only partially by those of Yoshida sarcoma, of pig spleen and of calf thymus, the sarcoma DNA resembling in this respect those of tissues of animals differ-

TABLE III

Complement Fixing Test of the Antisera of Spleen and Liver of Rat and Yoshida Sarcoma together with Saline Solutions of the Corresponding Tissues

−: no hemolysis, +∼卌: slight to complete hemolysis. The sera collected from the rabbits prior to immunization showed no complement fixation. Different antiserum samples of the same tissue gave similar results, and hence one example only is shown.

Antiserum (0.25 cc.)	In vitro antigen*	Dilution of the antisera (1:)										
		10	2× 10	4× 10	8× 10	1.6× 10^2	3.2× 10^2	6.4× 10^2	1.28 ×10^3	2.56 ×10^3	5.12 ×10^3	∞
Anti-rat spleen serum M_3	rat spleen (16-fold diluted)	−	−	−	−	−	−	−	++	卌	卌	卌
Physiol. saline		卌	卌	卌	卌	卌	卌	卌	卌	卌	卌	
Anti-rat liver serum L_2	rat liver (8-fold diluted)	−	−	−	−	−	−	+	卌	卌	卌	卌
Physiol. saline		卌	卌	卌	卌	卌	卌	卌	卌	卌	卌	
Anti-Yoshida sarcoma Y_3	Yoshida sarcoma (8-fold diluted)	−	−	−	−	−	−	−	++	卌	卌	卌
Physiol. saline		卌	卌	卌	卌	卌	卌	卌	卌	卌	卌	

* Tissue solutions in 9 volumes of physiol. saline containing 0.5% phenol (centrifuged supernatants) were diluted as written in parentheses and 0.25 cc. portions of the diluted solutions were employed for the assay.

ent from rat. DNAs from pig spleen and Yoshida sarcoma could absorb the antibody till no more positive precipitin reaction of them and calf thymus DNA but not of DNAs of rat normal tissues, and calf thymus DNA could absorb only the partial antibodies reacting with itself and pig spleen DNA.

4) In Table VII showing experiments similar to above on the anti-rat liver serum, the results resembled those in 3), excepting that, in this case, calf thymus DNA behaved as to absorption of the antiserum like pig spleen- and Yoshida sarcoma-DNA.

5) As is seen from Table VIII, DNAs of rat normal tissues absorbed the antibody reacting with the various DNAs from the anti-Yoshida sarcoma serum entirely. DNAs of pig spleen and calf thymus of the same amount as the ones above could absorb the partial antibodies reacting with the DNAs of the other tissues including Yoshida sarcoma only in part. However, more of them could effect complete absorption. It is noteworthy that here the DNAs of the normal rat tissues as well as those of pig and calf tissues could eliminate the precipitin of Yoshida sarcoma DNA most difficultly among those of the other DNAs from the anti-Yo-

TABLE IV

Complement Eixation Test of the Antisera of Rat Spleen, Rat Liver and Yoshida Sarcoma together with the DNAs from those Tissues and Rat Thymus

− : no hemolysis, ±~卌 : faint to complete hemolysis.

Antiserum*,† of	Antigen‡: DNA of	Dilution of the antigen solutions (1:)							
		1	2	4	8	1.6×10	3.2×10	6.4×10	∞
Rat spleen (×40)	rat spleen	卌	卌	卌	卌	卌	卌	卌	卌
Rat liver (×20)		+	卌	卌	卌	卌	卌	卌	卌
Yoshida sarcoma (×40)		+	卌	卌	卌	卌	卌	卌	卌
Physiol. saline		+	卌	卌	卌	卌	卌	卌	
Rat spleen (×40)	rat liver	−	±	卄	卄	卌	卌	卌	
Rat liver (×20)		−	±	+	+	卌	卌	卌	
Yoshida sarcoma (×40)		±	+	+	卄	卄	卌	卌	
Physiol. saline		+	+	卄	卄	卌	卌	卌	
Rat spleen (×40)	rat thymus	−	−	±	+	卄	卌	卌	
Rat liver (×20)		−	−	±	+	卌	卌	卌	
Yoshida sarcoma (×40)		−	−	+	卄	卌	卌	卌	
Physiol. saline		−	−	−	卄	卄	卌	卌	
Rat spleen (×40)	Yoshida sarcoma	−	+	卄	卌	卌	卌	卌	
Rat liver (×20)		−	+	卄	卌	卌	卌	卌	
Yoshida sarcoma (×40)		−	卄	卌	卌	卌	卌	卌	
Physiol. saline		−	−	卄	卌	卌	卌	卌	

* Same samples that were used in the experiment in Table III. † The antisera were diluted as written in parentheses and 0.25 cc. portions of the diluted solutions were used for the test. ‡ Dissolved in 10 volumes of physiol. saline and 0.25 cc. portions of the solutions were used as the original solutions for the assay.

shida sarcoma serum, namely, the sarcoma DNA must have a structure somewhat peculiar.

6) Of the various DNAs, rat thymus DNA absorbed generally the DNA-antibody from the antisera most readily, and on the other hand, calf thymus DNA absorbed the partial antibody reacting with rat thymus DNA more readily than those reacting with rat spleen- and liver-DNA. It thus appeared that thymus DNA is richer in both of species-specific and organ-specific structures in comparison with spleen- and liver-DNA.

7) As to the deviation of Yoshida sarcoma DNA from those of rat

TABLE V

Precipitin Reaction of the Various DNAs with the Antisera

The antisera were used without dilution. −: negative, ±: trace, +: positive. No reaction occurred when the antisera were replaced with blood serum samples of the rabbits before immunization.

Antiserum* of	Antigen: DNA of	Dilution of antigen solutions† (1:)										
		2×10	4×10	8×10	1.6×10^2	3.2×10^2	6.4×10^2	1.28×10^3	2.56×10^3	5.12×10^3	1.024×10^4	∞
Rat spleen	rat spleen	+	+	+	+	+	+	+	+	+	−	−
	» liver	+	+	+	+	+	+	+	−	−	−	
	» thymus	+	+	+	+	+	+	−	−	−	−	
	Yoshida sarcoma	+	+	+	+	−	−	−	−	−	−	
	pig spleen	+	+	+	+	−	−	−	−	−	−	
	calf thymus	+	+	+	−	−	−	−	−	−	−	
Rat liver	rat spleen	+	+	+	+	+	+	+	±	−	−	−
	» liver	+	+	+	+	+	+	+	−	−	−	
	» thymus	+	+	+	+	+	+	−	−	−	−	
	Yoshida sarcoma	+	+	+	+	−	−	−	−	−	−	
	pig spleen	+	+	+	−	−	−	−	−	−	−	
	calf thymus	+	+	−	−	−	−	−	−	−	−	
Yoshida sarcoma	rat spleen	+	+	+	+	+	+	±	−	−	−	−
	» liver	+	+	+	+	+	−	−	−	−	−	
	» thymus	+	+	+	+	±	−	−	−	−	−	
	Yoshida sarcoma	+	+	+	+	+	+	+	+	−	−	
	pig spleen	+	+	+	−	−	−	−	−	−	−	
	calf thymus	+	+	+	−	−	−	−	−	−	−	

* Same samples that were used in the experiment in Table III. † Original solutions were prepared by dissolving the DNAs in 10 volumes of physiol. saline.

normal tissues, the facts described in 3)–4) suggest some resemblance of it with the DNAs of tissues of animals different from rat, and the facts in 5) the resemblance of it with the DNAs of normal rat tissues and also some peculiarity of it. Hence it is plausibly assumed that the sarcoma possesses a DNA of a structure somewhat characteristic of itself with decreased species-specificity. Such, we believe, is in general the immature configuration of high-molecular, species-specific tissue components, which undergo no marked alteration of the quantitative composition, qualitatively being unaltered, when a tissue changes malignant.

SUMMARY

DNAs of rat normal tissues, Yoshida sarcoma, pig spleen and calf

TABLE VI

Precipitin Reaction of the Various DNAs with Portions of the Anti-Rat Spleen Serum* Absorbed with those DNAs

The antiserum was absorbed after dilution two times. −: negative, ∓: faint trace, ±: trace, +: positive.

Amount of DNAs used for absorption in mg.	Antigen for precipitin test (source)	Dilution of the antigen solutions† (1:)										
		2× 10	4× 10	8× 10	1.6× 10^2	3.2× 10^2	6.4× 10^2	1.28 ×10^3	2.56 ×10^3	5.12 ×10^3	1.024 ×10^4	∞
A. The antiserum was absorbed with rat-liver DNA												
0‡	rat spleen	+	+	+	+	+	+	+	+	+	−	−
	" liver	+	+	+	+	+	+	+	−	−	−	
	" thymus	+	+	+	+	+	+	−	−	−	−	
	Yoshida sarc.	+	+	+	+	−	−	−	−	−	−	
	pig spleen	+	+	+	+	−	−	−	−	−	−	
	calf thymus	+	+	+	−	−	−	−	−	−	−	
2	rat spleen	+	+	−	−	−	−	−	−	−	−	−
	" liver	+	−	−	−	−	−	−	−	−	−	
	" thymus	+	+	−	−	−	−	−	−	−	−	
	Yoshida sarc.	+	+	−	−	−	−	−	−	−	−	
	pig spleen	+	±	−	−	−	−	−	−	−	−	
	calf thymus	−	−	−	−	−	−	−	−	−	−	
3	rat spleen	−	−	−	−	−	−	−	−	−	−	−
	" liver	−	−	−	−	−	−	−	−	−	−	
	" thymus	−	−	−	−	−	−	−	−	−	−	
	Yoshida sarc.	−	−	−	−	−	−	−	−	−	−	
	pig spleen	−	−	−	−	−	−	−	−	−	−	
	calf thymus	−	−	−	−	−	−	−	−	−	−	
B. The antiserum was absorbed with rat-thymus DNA												
2	rat spleen	−	−	−	−	−	−	−	−	−	−	−
	" liver	−	−	−	−	−	−	−	−	−	−	
	" thymus	∓	−	−	−	−	−	−	−	−	−	
	Yoshida sarc.	−	−	−	−	−	−	−	−	−	−	
	pig spleen	−	−	−	−	−	−	−	−	−	−	
	calf thymus	−	−	−	−	−	−	−	−	−	−	
C. The antiserum was absorbed with Yoshida-sarcoma DNA												
2	rat spleen	+	+	+	+	+	+	+	+	−	−	−
	" liver	+	+	+	+	+	+	+	−	−	−	
	" thymus	+	+	+	+	±	−	−	−	−	−	
	Yoshida sarc.	+	−	−	−	−	−	−	−	−	−	
	pig spleen	+	+	+	−	−	−	−	−	−	−	
	calf thymus	+	−	−	−	−	−	−	−	−	−	
3	rat spleen	+	+	+	−	−	−	−	−	−	−	−
	" liver	+	+	+	−	−	−	−	−	−	−	
	" thymus	+	+	+	±	−	−	−	−	−	−	
	Yoshida sarc.	−	−	−	−	−	−	−	−	−	−	
	pig spleen	+	±	−	−	−	−	−	−	−	−	
	calf thymus	−	−	−	−	−	−	−	−	−	−	
5	rat spleen	+	+	+	−	−	−	−	−	−	−	−
	" liver	+	+	+	−	−	−	−	−	−	−	
	" thymus	+	+	±	−	−	−	−	−	−	−	
	Yoshida sarc.	−	−	−	−	−	−	−	−	−	−	
	pig spleen	−	−	−	−	−	−	−	−	−	−	
	calf thymus	−	−	−	−	−	−	−	−	−	−	

TABLE VI—*Concluded*

Amount of DNAs used for absorption in mg.	Antigen for precipitin test (source)	Dilution of the antigen solutions† (1 :)										
		2× 10	4× 10	8× 10	1.6× 10^2	3.2× 10^2	6.4× 10^2	1.28 ×10^3	2.56 ×10^3	5.12 ×10^3	1.024 ×10^4	∞
D. The antiserum was absorbed with pig-spleen DNA												
2	rat spleen	+	+	+	+	+	−	−	−	−	−	−
	» liver	+	+	+	+	+	+	−	−	−	−	
	» thymus	+	+	+	+	−	−	−	−	−	−	
	Yoshida sarc.	+	±	−	−	−	−	−	−	−	−	
	pig spleen	∓	−	−	−	−	−	−	−	−	−	
	calf thymus	±	−	−	−	−	−	−	−	−	−	
3	rat spleen	+	+	+	−	−	−	−	−	−	−	−
	» liver	+	+	+	±	−	−	−	−	−	−	
	» thymus	+	+	±	−	−	−	−	−	−	−	
	Yoshida sarc.	±	−	−	−	−	−	−	−	−	−	
	pig spleen	−	−	−	−	−	−	−	−	−	−	
	calf thymus	±	−	−	−	−	−	−	−	−	−	
5	rat spleen	+	+	+	−	−	−	−	−	−	−	−
	» liver	+	+	+	∓	−	−	−	−	−	−	
	» thymus	+	+	±	−	−	−	−	−	−	−	
	Yoshida sarc.	−	−	−	−	−	−	−	−	−	−	
	pig spleen	−	−	−	−	−	−	−	−	−	−	
	calf thymus	−	−	−	−	−	−	−	−	−	−	
E. The antiserum was absorbed with calf-thymus DNA												
2	rat spleen	+	+	+	+	+	+	−	−	−	−	−
	» liver	+	+	+	+	+	+	−	−	−	−	
	» thymus	+	+	−	−	−	−	−	−	−	−	
	Yoshida sarc.	+	+	−	−	−	−	−	−	−	−	
	pig spleen	+	−	−	−	−	−	−	−	−	−	
	calf thymus	−	−	−	−	−	−	−	−	−	−	
3	rat spleen	+	+	+	+	∓	−	−	−	−	−	−
	» liver	+	+	+	+	−	−	−	−	−	−	
	» thymus	+	+	−	−	−	−	−	−	−	−	
	Yoshida sarc.	+	+	−	−	−	−	−	−	−	−	
	pig spleen	−	−	−	−	−	−	−	−	−	−	
	calf thymus	−	−	−	−	−	−	−	−	−	−	
5	rat spleen	+	+	+	+	−	−	−	−	−	−	−
	» liver	+	+	+	+	−	−	−	−	−	−	
	» thymus	+	+	−	−	−	−	−	−	−	−	
	Yoshida sarc.	+	±	−	−	−	−	−	−	−	−	
	pig spleen	−	−	−	−	−	−	−	−	−	−	
	calf thymus	−	−	−	−	−	−	−	−	−	−	

* Same sample that was used in the experiment in Table III. † The original solutions were prepared by dissolving the DNAs in 10 volumes of physiol. saline. ‡ A part of Table V is reproduced.

TABLE VII

Precipitin Reaction of the Various DNAs with Portions of the Anti-Rat Liver Serum* Absorbed with those DNAs

The antiserum was absorbed after dilution two times. − : negative, ∓ : faint trace, ± : trace, + : positive.

Amount of DNAs used for absorption in mg.	Antigen (DNA) for precipitin test (source)	Dilution of the antigen solutions† (1 :)										
		2× 10	4× 10	8× 10	1.6× 10^2	3.2× 10^2	6.4× 10^2	1.28 ×10^3	2.56 ×10^3	5.12 ×10^3	1.024 ×10^4	∞
A. The antiserum was absorbed with rat-spleen DNA												
0‡	rat spleen	+	+	+	+	+	+	+	±	−	−	−
	» liver	+	+	+	+	+	+	+	−	−	−	
	» thymus	+	+	+	+	+	+	−	−	−	−	
	Yoshida sarc.	+	+	+	+	−	−	−	−	−	−	
	pig spleen	+	+	+	−	−	−	−	−	−	−	
	calf thymus	+	+	−	−	−	−	−	−	−	−	
2	rat spleen	±	−	−	−	−	−	−	−	−	−	−
	» liver	±	−	−	−	−	−	−	−	−	−	
	» thymus	+	±	−	−	−	−	−	−	−	−	
	Yoshida sarc.	+	−	−	−	−	−	−	−	−	−	
	pig spleen	−	−	−	−	−	−	−	−	−	−	
	calf thymus	−	−	−	−	−	−	−	−	−	−	
3	rat spleen	−	−	−	−	−	−	−	−	−	−	−
	» liver	−	−	−	−	−	−	−	−	−	−	
	» thymus	±	−	−	−	−	−	−	−	−	−	
	Yoshida sarc.	−	−	−	−	−	−	−	−	−	−	
	pig spleen	−	−	−	−	−	−	−	−	−	−	
	calf thymus	−	−	−	−	−	−	−	−	−	−	
B. The antiserum was absorbed with rat-thymus DNA												
2	rat spleen	−	−	−	−	−	−	−	−	−	−	−
	» liver	−	−	−	−	−	−	−	−	−	−	
	» thymus	±	−	−	−	−	−	−	−	−	−	
	Yoshida sarc.	−	−	−	−	−	−	−	−	−	−	
	pig spleen	−	−	−	−	−	−	−	−	−	−	
	calf thymus	−	−	−	−	−	−	−	−	−	−	
C. The antiserum was absorbed with Yoshida-sarcoma DNA												
2	rat spleen	+	+	+	+	+	+	−	−	−	−	−
	» liver	+	+	+	+	+	+	−	−	−	−	
	» thymus	+	+	+	+	+	+	−	−	−	−	
	Yoshida sarc.	+	−	−	−	−	−	−	−	−	−	
	pig spleen	+	+	−	−	−	−	−	−	−	−	
	calf thymus	−	−	−	−	−	−	−	−	−	−	
3	rat spleen	+	+	+	±	−	−	−	−	−	−	−
	» liver	+	+	+	−	−	−	−	−	−	−	
	» thymus	+	+	+	±	−	−	−	−	−	−	
	Yoshida sarc.	−	−	−	−	−	−	−	−	−	−	
	pig spleen	+	−	−	−	−	−	−	−	−	−	
	calf thymus	−	−	−	−	−	−	−	−	−	−	
5	rat spleen	+	+	+	−	−	−	−	−	−	−	−
	» liver	+	+	+	−	−	−	−	−	−	−	
	» thymus	+	+	+	±	−	−	−	−	−	−	
	Yoshida sarc.	−	−	−	−	−	−	−	−	−	−	
	pig spleen	−	−	−	−	−	−	−	−	−	−	
	calf thymus	−	−	−	−	−	−	−	−	−	−	

TABLE VII—*Concluded*

Amount of DNAs used for absorption in mg.	Antigen (DNA) for precipitin test (source)	Dilution of the antigen solutions† (1 :)										
		2× 10	4× 10	8× 10	1.6× 10^2	3.2× 10^2	6.4× 10^2	1.28 ×10^3	2.56 ×10^3	5.12 ×10^3	1.024 ×10^4	∞
D. The antiserum was absorbed with pig-spleen DNA												
2	rat spleen	+	+	+	+	+	+	−	−	−	−	−
	» liver	+	+	+	+	+	+	−	−	−	−	
	» thymus	+	+	+	−	−	−	−	−	−	−	
	Yoshida sarc.	+	−	−	−	−	−	−	−	−	−	
	pig spleen	−	−	−	−	−	−	−	−	−	−	
	calf thymus	−	−	−	−	−	−	−	−	−	−	
3	rat spleen	+	+	+	±	−	−	−	−	−	−	−
	» liver	+	+	+	+	−	−	−	−	−	−	
	» thymus	+	+	±	−	−	−	−	−	−	−	
	Yoshida sarc.	+	−	−	−	−	−	−	−	−	−	
	pig spleen	−	−	−	−	−	−	−	−	−	−	
	calf thymus	−	−	−	−	−	−	−	−	−	−	
5	rat spleen	+	+	+	−	−	−	−	−	−	−	−
	» liver	+	+	+	±	−	−	−	−	−	−	
	» thymus	+	+	+	−	−	−	−	−	−	−	
	Yoshida sarc.	±	−	−	−	−	−	−	−	−	−	
	pig spleen	−	−	−	−	−	−	−	−	−	−	
	calf thymus	−	−	−	−	−	−	−	−	−	−	
E. The antiserum was absorbed with calf-thymus DNA												
2	rat spleen	+	+	+	+	+	−	−	−	−	−	−
	» liver	+	+	+	+	+	±	−	−	−	−	
	» thymus	+	+	+	±	−	−	−	−	−	−	
	Yoshida sarc.	+	−	−	−	−	−	−	−	−	−	
	pig spleen	+	−	−	−	−	−	−	−	−	−	
	calf thymus	−	−	−	−	−	−	−	−	−	−	
3	rat spleen	+	+	+	−	−	−	−	−	−	−	−
	» liver	+	+	±	−	−	−	−	−	−	−	
	» thymus	+	+	−	−	−	−	−	−	−	−	
	Yoshida sarc.	±	−	−	−	−	−	−	−	−	−	
	pig spleen	−	−	−	−	−	−	−	−	−	−	
	calf thymus	−	−	−	−	−	−	−	−	−	−	
5	rat spleen	+	+	∓	−	−	−	−	−	−	−	−
	» liver	+	+	∓	−	−	−	−	−	−	−	
	» thymus	+	±	−	−	−	−	−	−	−	−	
	Yoshida sarc.	∓	−	−	−	−	−	−	−	−	−	
	pig spleen	−	−	−	−	−	−	−	−	−	−	
	calf thymus	−	−	−	−	−	−	−	−	−	−	

* Same sample that was used in the experiment in Table III. † The original solutions were prepared by dissolving the DNAs in 10 volumes of physiol. saline. ‡A part of Table V is reproduced.

TABLE VIII

Precipitin Reaction of the Various DNAs with Portions of the Anti-Yoshida Sarcoma Serum* Absorbed with those DNAs

The antiserum was absorbed after dilution two times. − : negative, ∓ : faint trace, ± : trace, + : positive.

Amount of DNAs used for absorption in mg.	Antigen (DNA) for precipitin test (source)	Dilution of the antigen solutions† (1 :)										
		2× 10	4× 10	8× 10	1.6× 10^2	3.2× 10^2	6.4× 10^2	1.28 ×10^3	2.56 ×10^3	5.12 ×10^3	1.024 ×10^4	∞
A. The antiserum was absorbed with rat-spleen DNA												
0‡	rat spleen	+	+	+	+	+	+	±	−	−	−	−
	» liver	+	+	+	+	+	−	−	−	−	−	
	» thymus	+	+	+	+	±	−	−	−	−	−	
	Yoshida sarc.	+	+	+	+	+	+	+	+	−	−	
	pig spleen	+	+	+	−	−	−	−	−	−	−	
	calf thymus	+	+	+	−	−	−	−	−	−	−	
2	rat spleen	−	−	−	−	−	−	−	−	−	−	−
	» liver	±	−	−	−	−	−	−	−	−	−	
	» thymus	+	+	−	−	−	−	−	−	−	−	
	Yoshida sarc.	+	+	+	−	−	−	−	−	−	−	
	pig spleen	−	−	−	−	−	−	−	−	−	−	
	calf thymus	+	−	−	−	−	−	−	−	−	−	
3	rat spleen	−	−	−	−	−	−	−	−	−	−	−
	» liver	−	−	−	−	−	−	−	−	−	−	
	» thymus	±	−	−	−	−	−	−	−	−	−	
	Yoshida sarc.	+	+	±	−	−	−	−	−	−	−	
	pig spleen	−	−	−	−	−	−	−	−	−	−	
	calf thymus	−	−	−	−	−	−	−	−	−	−	
5	rat spleen	−	−	−	−	−	−	−	−	−	−	−
	» liver	−	−	−	−	−	−	−	−	−	−	
	» thymus	−	−	−	−	−	−	−	−	−	−	
	Yoshida sarc.	−	−	−	−	−	−	−	−	−	−	
	pig spleen	−	−	−	−	−	−	−	−	−	−	
	calf thymus	−	−	−	−	−	−	−	−	−	−	
B. The antiserum was absorbed with rat-liver DNA												
2	rat spleen	+	−	−	−	−	−	−	−	−	−	−
	» liver	+	−	−	−	−	−	−	−	−	−	
	» thymus	+	±	−	−	−	−	−	−	−	−	
	Yoshida sarc.	+	+	+	−	−	−	−	−	−	−	
	pig spleen	−	−	−	−	−	−	−	−	−	−	
	calf thymus	+	±	−	−	−	−	−	−	−	−	
3	rat spleen	−	−	−	−	−	−	−	−	−	−	−
	» liver	−	−	−	−	−	−	−	−	−	−	
	» thymus	−	−	−	−	−	−	−	−	−	−	
	Yoshida sarc.	+	+	±	−	−	−	−	−	−	−	
	pig spleen	−	−	−	−	−	−	−	−	−	−	
	calf thymus	±	−	−	−	−	−	−	−	−	−	
5	rat spleen	−	−	−	−	−	−	−	−	−	−	−
	» liver	−	−	−	−	−	−	−	−	−	−	
	» thymus	−	−	−	−	−	−	−	−	−	−	
	Yoshida sarc.	±	−	−	−	−	−	−	−	−	−	
	pig spleen	−	−	−	−	−	−	−	−	−	−	
	calf thymus	−	−	−	−	−	−	−	−	−	−	

TABLE VIII—*Continued*

Amount of DNAs used for absorption in mg.	Antigen (DNA) for precipitin test (source)	Dilution of the antigen solutions† (1 :)										
		2×10	4×10	8×10	1.6×10^2	3.2×10^2	6.4×10^2	1.28×10^3	2.56×10^3	5.12×10^3	1.024×10^4	∞
C. The antiserum was absorbed with rat-thymus DNA												
2	rat spleen	−	−	−	−	−	−	−	−	−	−	−
	» liver	+	−	−	−	−	−	−	−	−	−	
	» thymus	±	−	−	−	−	−	−	−	−	−	
	Yoshida sarc.	+	+	±	−	−	−	−	−	−	−	
	pig spleen	−	−	−	−	−	−	−	−	−	−	
	calf thymus	−	−	−	−	−	−	−	−	−	−	
3	rat spleen	−	−	−	−	−	−	−	−	−	−	−
	» liver	−	−	−	−	−	−	−	−	−	−	
	» thymus	−	−	−	−	−	−	−	−	−	−	
	Yoshida sarc.	+	−	−	−	−	−	−	−	−	−	
	pig spleen	−	−	−	−	−	−	−	−	−	−	
	calf thymus	−	−	−	−	−	−	−	−	−	−	
5	rat spleen	−	−	−	−	−	−	−	−	−	−	−
	» liver	−	−	−	−	−	−	−	−	−	−	
	» thymus	−	−	−	−	−	−	−	−	−	−	
	Yoshida sarc.	−	−	−	−	−	−	−	−	−	−	
	pig spleen	−	−	−	−	−	−	−	−	−	−	
	calf thymus	−	−	−	−	−	−	−	−	−	−	
D. The antiserum was absorbed with pig-spleen DNA												
2	rat spleen	+	+	−	−	−	−	−	−	−	−	−
	» liver	+	+	+	−	−	−	−	−	−	−	
	» thymus	+	+	∓	−	−	−	−	−	−	−	
	Yoshida sarc.	+	+	+	−	−	−	−	−	−	−	
	pig spleen	−	−	−	−	−	−	−	−	−	−	
	calf thymus	−	−	−	−	−	−	−	−	−	−	
3	rat spleen	+	+	−	−	−	−	−	−	−	−	−
	» liver	+	+	−	−	−	−	−	−	−	−	
	» thymus	+	+	−	−	−	−	−	−	−	−	
	Yoshida sarc.	+	+	∓	−	−	−	−	−	−	−	
	pig spleen	−	−	−	−	−	−	−	−	−	−	
	calf thymus	−	−	−	−	−	−	−	−	−	−	
5	rat spleen	+	−	−	−	−	−	−	−	−	−	−
	» liver	+	−	−	−	−	−	−	−	−	−	
	» thymus	+	−	−	−	−	−	−	−	−	−	
	Yoshida sarc.	+	±	−	−	−	−	−	−	−	−	
	pig spleen	−	−	−	−	−	−	−	−	−	−	
	calf thymus	−	−	−	−	−	−	−	−	−	−	
7	rat spleen	−	−	−	−	−	−	−	−	−	−	−
	» liver	−	−	−	−	−	−	−	−	−	−	
	» thymus	−	−	−	−	−	−	−	−	−	−	
	Yoshida sarc.	−	−	−	−	−	−	−	−	−	−	
	pig spleen	−	−	−	−	−	−	−	−	−	−	
	calf thymus	−	−	−	−	−	−	−	−	−	−	

TABLE VIII—*Concluded*

Amount of DNAs used for absorption in mg.	Antigen (DNA) for precipitin test (source)	Dilution of the antigen solutions† (1 :)										
		2× 10	4× 10	8× 10	1.6× 10^2	3.2× 10^2	6.4× 10^2	1.28 ×10^3	2.56 ×10^3	5.12 ×10^3	1.024 ×10^4	∞
E. The antiserum was absorbed with calf-thymus DNA												
2	rat spleen	+	+	+	+	−	−	−	−	−	−	−
	» liver	+	+	+	−	−	−	−	−	−	−	
	» thymus	+	+	±	−	−	−	−	−	−	−	
	Yoshida sarc.	+	+	+	+	−	−	−	−	−	−	
	pig spleen	+	−	−	−	−	−	−	−	−	−	
	calf thymus	−	−	−	−	−	−	−	−	−	−	
3	rat spleen	+	+	−	−	−	−	−	−	−	−	−
	» liver	+	+	−	−	−	−	−	−	−	−	
	» thymus	+	±	−	−	−	−	−	−	−	−	
	Yoshida sarc.	+	+	±	−	−	−	−	−	−	−	
	pig spleen	−	−	−	−	−	−	−	−	−	−	
	calf thymus	−	−	−	−	−	−	−	−	−	−	
5	rat spleen	+	−	−	−	−	−	−	−	−	−	−
	» liver	+	−	−	−	−	−	−	−	−	−	
	» thymus	±	−	−	−	−	−	−	−	−	−	
	Yoshida sarc.	+	−	−	−	−	−	−	−	−	−	
	pig spleen	−	−	−	−	−	−	−	−	−	−	
	calf thymus	−	−	−	−	−	−	−	−	−	−	
7	rat spleen	−	−	−	−	−	−	−	−	−	−	−
	» liver	−	−	−	−	−	−	−	−	−	−	
	» thymus	−	−	−	−	−	−	−	−	−	−	
	Yoshida sarc.	−	−	−	−	−	−	−	−	−	−	
	pig spleen	−	−	−	−	−	−	−	−	−	−	
	calf thymus	−	−	−	−	−	−	−	−	−	−	

* Same sample that was used in the experiment in Table III. † The original solutions were prepared by dissolving the DNAs in 10 volumes of physiol. saline. ‡ A part of Table V is reproduced.

thymus were investigated chemically and immunologically, and species- and organ-specificity and immaturity of DNAs are contemplated.

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On Free Amino Acids of Human Gastric Juice

By

Hirosi Tiba and **Sumio Isikawa**
(千葉　博)　　(石川 澄雄)

From the Medical Clinic of Prof. T. Kurokawa, Tohoku University, Sendai

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We have made quantitative determinations on non-protein nitrogenous compounds in gastric juice, particularly of patients with gastric lesions.

In 1950 Cagianut[1] and his cooperators confirmed the presence of free amino acids in human gastric juice by paper partition chromatography, in addition to glucosamine and histamine. In 1951 Gilligan[2] and his cooperators noted that amino acids in gastric juice did not vary from the normal in the case of gastric and duodenal ulcer in quantitative as well as in qualitative aspect, but remarkably increased in the case of gastric cancer.

Experimental

1. *Analysis of non-protein nitrogenous compound*

Gastric juice from patients was filtrated through a creased paper. To 3 cc. of the clear filtrate, 2 volumes of 8.6% perchloric acid were added, and heated it on a water-bath at 80°C for 3 minutes and rejected white precipitates that appeared. The fluid gave the negative biuret reaction but the positive ninhydrin reaction. Because of high mucin content, gastric juice is difficult to be freed completely from protein by most former method, but perchloric acid served well the purpose. We classified gastric juice according to 5 groups of patients—nongastric diseases, chronic gastritis, gastric ulcer, duodenal ulcer and gastric cancer—and estimated total and free amino nitrogen according to Kjeldahl and van Slyke.

The results of estimation are given in Tables I to V.

Among 5 groups, statistically significant difference was examined by means of total-N, free amino-N and amino index.

In the case of gastric cancer total-N increased more remarkably than in other 4 groups, among which no significant difference was noticed. (Fig. 1)

Amino-N did not increase in the case of chronic gastritis but it increased in the case of gastric ulcer and duodenal ulcer more than in the

TABLE I

Non-gastric Diseases

No. of case	Sex	Age		Acidity	Occult Blood	Total-N† mg/dl	Free Amino-N‡ mg/dl	Amino-index $\frac{\text{Amino-N}}{\text{Total-N}} \times 100$
*1	F	28	Pulmonary Tuberculosis	Hypo-	—	20.15	13.26	65.80
*2	M	49	Cancer of liver	Hypo-	—	19.53	18.98	97.28
*3	F	56	Ascariasis	Norm-	—	19.56	15.28	77.12
4	F	34	Cholecystopathy	Hypo-	—	30.45	19.05	62.66
*5	M	31	Chronic appendicitis	Norm-	—	17.39	14.22	81.87
6	F	28	Beriberi	Norm-	—	21.36	17.46	81.74
7	F	38	Cholecystopathy	Hypo-	—	28.96	24.33	84.01
8	M	39	Bronchial asthma	Norm-	—	22.32	17.68	79.21
					Mean	22.47	17.53	78.71

* Paper partition chromatography as amino acid components was performed.
† Micro-Kjeldahl.
‡ Micro van Slyke.

TABLE II

Chronic Gastritis

No. of case	Sex	Age	Acidity	Occult Blood	Total-N† mg/dl	Free Amino-N‡ mg/dl	Amino-index $\frac{\text{Amino-N}}{\text{Total-N}} \times 10$
* 1	F	33	Hyper-	—	20.46	16.72	81.72
* 2	M	52	Hyper-	—	25.62	18.90	73.77
* 3	M	42	Norm-	—	23.50	13.70	58.29
* 4	M	15	Hyper-	—	22.30	17.82	79.91
* 5	M	29	Hypo-	+	28.63	20.86	72.86
6	F	18	Hypo-	—	21.21	17.81	83.96
7	M	47	Hyper-	+	36.12	27.49	76.10
8	F	27	Hyper-	—	24.78	15.47	62.42
* 9	F	34	An-	+	29.93	18.90	63.14
*10	M	38	An-	—	30.65	24.68	80.52
11	M	29	An-	—	23.23	18.60	80.00
12	F	36	Hypo-	—	20.16	16.49	81.29
13	M	46	Hypo-	—	26.43	20.10	76.05
				Mean	25.62	19.04	74.62

*, † and ‡ signify the same as in Table I.

case of non-gastric diseases. Especially in the case of gastric cancer,more noticeable increase of amino-N, was noted than in other 4 groups, but no

TABLE III

Gastric Ulcer

No. of case	Sex	Age	Acidity	Occult Blood	Total-N† mg/dl	Free Amino-N‡ mg/dl	Amino-index $\frac{\text{Amino-N}}{\text{Total-N}} \times 100$
* 1	M	24	Norm-	+	24.15	22.92	94.90
* 2	M	37	Hyper-	+	31.29	25.67	82.03
* 3	M	36	Hyper-	−	15.54	13.77	88.61
* 4	M	27	Norm-	−	16.38	13.97	85.28
* 5	M	32	Hypo-	++	29.40	25.87	87.99
* 6	M	20	Hyper-	++	27.93	25.76	92.23
* 7	M	42	Hyper-	+	22.38	19.62	87.66
* 8	M	65	An-	++	26.32	21.89	83.17
9	M	21	Hyper-	−	23.70	21.10	89.03
10	M	43	Hyper-	+	23.63	19.60	82.95
11	M	33	Hyper-	+	32.31	26.82	83.01
12	M	49	Hyper-	++	30.28	27.27	90.06
12 (10 days after)				++	28.61	25.36	88.64
12 (35 days after)				−	24.30	20.19	83.05
				Mean	25.27	22.02	87.24

*, † and ‡ signify the same as in Table I.

TABLE IV

Duodenal Ulcer

No. of case	Sex	Age	Acidity	Occult Blood	Total-N† mg/dl	Free Amino-N‡ mg/dl	Amino-index $\frac{\text{Amino-N}}{\text{Total-N}} \times 100$
* 1	M	48	Norm-	++	32.63	28.21	86.45
* 2	F	24	Hypo-	+	21.0	16.96	80.76
* 3	M	47	Hyper-	+	31.05	24.40	81.80
* 4	M	23	Norm-	+	27.0	20.76	76.88
5	M	42	Hyper-	+	26.82	22.61	84.30
6	M	50	Hyper-	+	25.33	20.85	82.31
7	M	36	Norm-	−	21.38	18.69	87.41
* 8	M	22	Hyper-	++	27.54	25.97	94.29
* 8 (20 days after)				+	24.78	17.86	72.07
9	M	32	Hyper-	+++	29.82	22.72	76.19
9 (30 days after)				+	19.50	17.31	88.76
				Mean	26.95	22.35	83.38

*, † and ‡ signify the same as in Table I.

TABLE V

Gastric Cancer

No. of case	Sex	Age	Acidity	Occult Blood	Total-N† mg/dl	Free Amino-N‡ mg/dl	Amino-index $\frac{\text{Amino-N}}{\text{Total-N}} \times 100$	Note of Rontgenogram
* 1	M	77	An-	##	48.09	39.36	81.84	dishlike form
* 2	M	57	An-	##	48.93	27.38	55.95	scirrhus
* 3	F	55	Hypo-	++	44.04	30.92	70.20	scirrhus
* 4	F	45	An-	++	53.34	43.45	81.45	scirrhus
* 5	M	50	Hypo-	##	55.86	46.49	83.22	dishlike form
* 6	M	50	An-	++	64.05	44.97	70.21	scirrhus
* 7	M	44	Hypo-	##	42.98	32.61	75.87	dishlike form
* 8	M	30	Hypo-	##	70.98	54.28	76.47	ulcerated form
* 9	M	16	Hypo-	++	54.10	43.36	80.14	dishlike form
10	M	53	An-	+	42.80	39.50	90.18	dishlike form
*11	M	48	An-	##	56.28	42.63	75.75	scirrhus
12	F	45	An-	##	65.31	45.87	70.24	dishlike form
13	M	59	An-	++	49.63	36.32	63.18	dishlike form
				Mean	53.57	40.55	74.98	

*, † and † signify the same as in Table I.

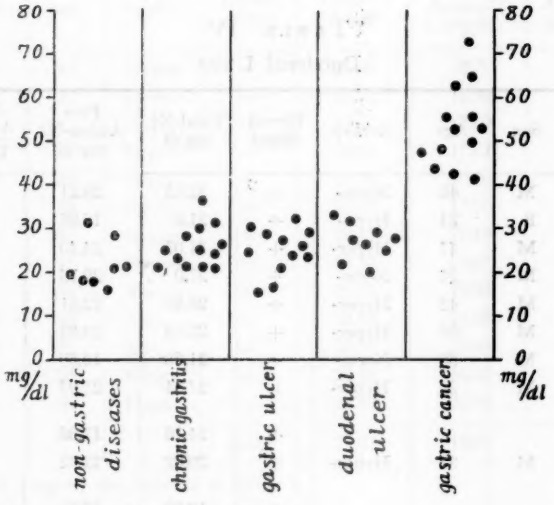

Fig. 1. Showing total-N (mg/dl).

significant difference was found among chronic gastritis, gastric ulcer and

duodenal ulcer. (Fig. 2)

Judging from amino index, amino acids largely increased in the case of gastric ulcer, but in gastric cancer much polypeptides were found besides amino acids. (Fig. 3)

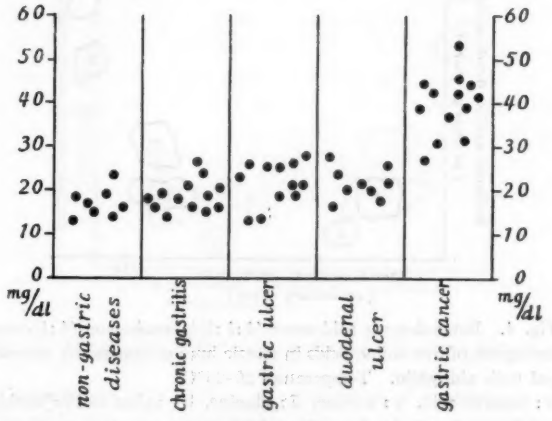

Fig. 2. Showing free amino-N (mg/dl).

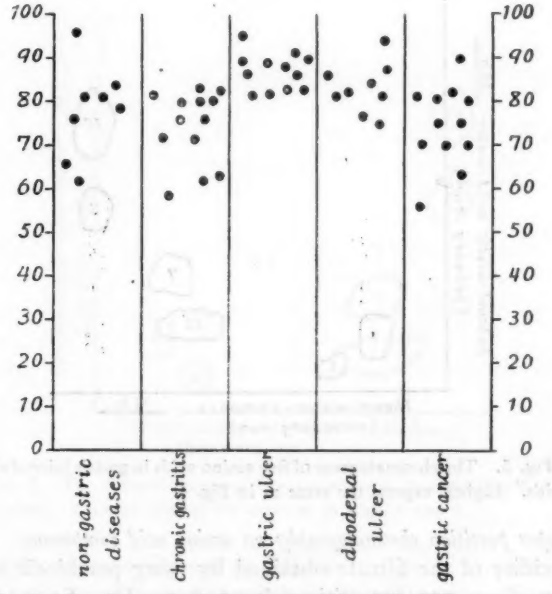

Fig. 3. Showing amino-index (amino-N/total-N × 100).

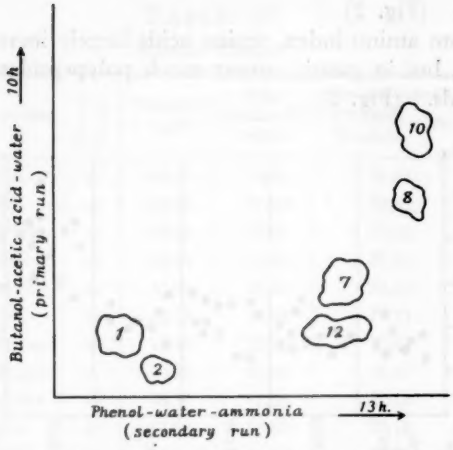

Fig. 4. Butanol-acetic acid-water (4 :1 :5)/phenol-water (4 :1)-ammonia chromatogram of free amino acids in gastric juice of non-gastric deseases, developed with ninhydrin. Temperature 20–23°C.

1 : aspartic acid, 2 : cystine, 7 : alanine, 8 : valine and/or methionine, 10 : leucine and/or isoleucine, 12 : histidine.

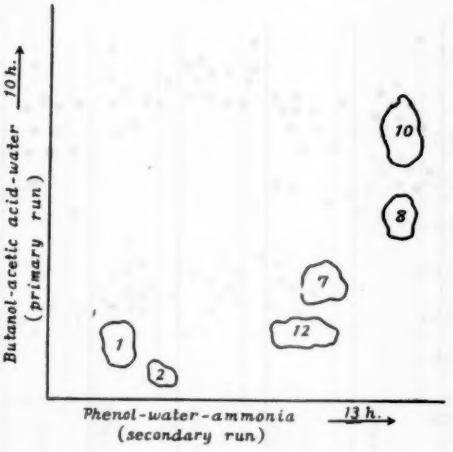

Fig. 5. The chromatogram of free amino acids in gastric juice of chronic gastritis. Ciphers express the same as in Fig. 4.

2. *Paper partition chromatography on amino acid components*

The acidity of the filtrate obtained by using perchloric acid was too intense to perform paper partition chromatography. So, paper partition

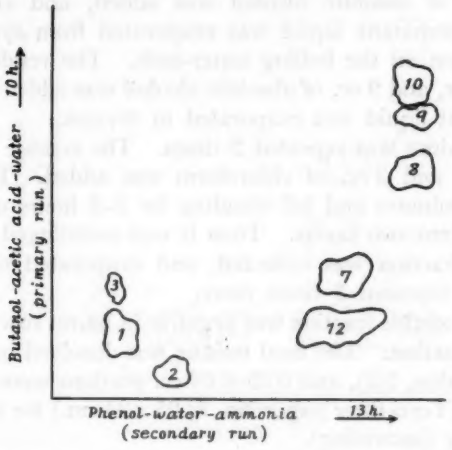

Fig. 6. The chromatogram of free amino acids in gastric juice of gastric ulcer.

1 : aspartic acid, 2 : cystine, 3 : cysteic acid, 7 : alanine, 8 : valine and/or methionine, 9 : phenylalamine, 10 : leucine and/or isoleucine, 12 : histidine.

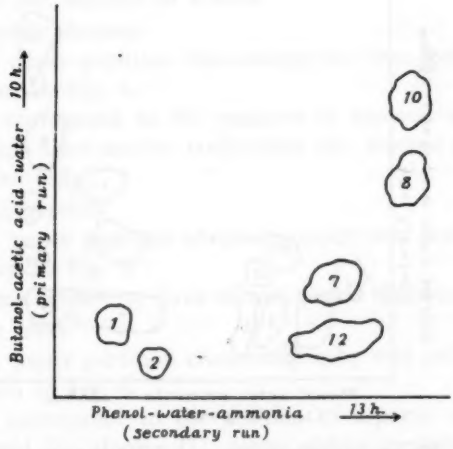

Fig. 7. The chromatogram of free amino acids in gastric juice of duodenal ulcer. Ciphers express the same as in Figs. 4 and 5.

chromatography was performed as follows:

Gastric juice was filtrated through a creased paper. To 2 cc. of the

filtrate, 18 cc. of absolute alcohol was added, and centrifuged. The transparent supernatant liquid was evaporated from syrupy consistency to almost dryness on the boiling water-bath. The residue was dissolved in 1 cc. of water, and 9 cc. of absolute alcohol was added and centrifuged. The supernatant liquid was evaporated to dryness.

This procedure was repeated 2 times. The residue was dissolved in 1 cc. of water, and 3 cc. of chloroform was added. The mixture was shaken for 30 minutes and left standing for 2–3 hours until the mixture was separated into two layers. Then it was centrifuged and the part of water soluble fraction was collected, and evaporated to dryness. This procedure was repeated 3 times more.

The water soluble fraction was negative in biuret reaction and positive in ninhydrin reaction. The final residue was dissolved in 0.3 cc. of water (pH of the solution, 5.6), and 0.05–0.07 cc. portions were put on a square piece of paper (Toyo filter paper No. 2, 25×24 cm.) for two -dimensional chromatography (ascending).

Primarily the spot was run with butanol-acetic acid-water (4 :1 :5). When the solvent advanced for almost 10 hours from the start, the paper was dried in air, and secondarily, it was run for almost 13 hours with phenol-water (4 :1) in the atmosphere saturated with ammonia. It was

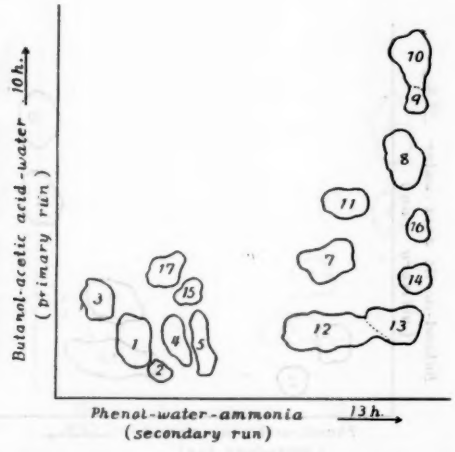

Fig. 8. The chromatogram of free amino acids in gastric juice of gastric cancer.

1 : aspartic acid, 2 : cystine, 3 : cysteic acid, 4 : glutamic acid, 5 : serine, 6 : glycine, 7 : alanine, 8 : valine and/or methionine, 9 : phenylalanine, 10 : leucine and/or isoleucine, 11 : tyrosine, 12 : histidine, 13 : arginine, 14 : proline, 15 : oxyproline, 16, 17 : two peptides.

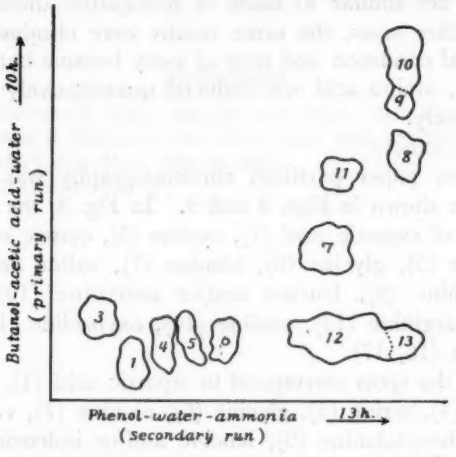

Fig. 9. The chromatogram of free amino acids in gastric juice of gastric cancer. Ciphers express the same as in Fig. 8.

dried and sprayed with the ninhydrin solution (0.2%), and heated. There appeared colored spots corresponding to the positions of amino acids.

Results are summarized as follows

1. Non-gastric diseases

In 4 cases, paper partition chromatography was performed, and one of them is shown in Fig. 4.

The spots correspond to the position of aspartic acid (1), cystine (2), alanine (7), valine and/or methionine (8), leucine and/or isoleucine (1) and histidine (12).

2. Chronic gastritis

In 7 cases, paper partition chromatography was performed, and one of them is shown in Fig. 5.

The spots are similar to those of non-gastric diseases.

3. Gastric ulcer

In 8 cases, paper partition chromatography was performed, and one of them is shown in Fig. 6.

The spots correspond to the position of aspartic acid (1), cystine (2), cysteic acid (3), alanine (7), valine and/or methionine (8), phenylalanine (9), leucine or isoleucine (10) and histidine (12).

4. Duodenal ulcer

In 6 cases, paper partition chromatography was performed, and one of them is shown in Fig. 7.

The spots are similar to those of non-gastric diseases and chronic gastritis. In other cases, the same results were obtained. In one case in which general condition and note of x-ray became better 20 days after our experiment, amino acid was reduced quantitatively but was not reduced qualitatively.

5. Gastric cancer

In 10 cases, paper partition chromatography was performed, and two of them are shown in Figs. 8 and 9. In Fig. 8, the spots correspond to the position of aspartic acid (1), cystine (2), cysteic acid (3), glutamic acid (4), serine (5), glycine (6), alanine (7), valine and/or methionine (8), phenylalanine (9), leucine and/or isoleucine (10), tyrosine (11), histidine (12), arginine (13), proline (14), oxyproline (15), and two unknown peptides (16, 17).

In Fig. 9, the spots correspond to aspartic acid (1), cysteic acid (3), glutamic acid (4), serine (5), glycine (6), alanine (7), valine and/or methionine (8), phenylalanine (9), leucine and/or isoleucine (10), tyrosine (11), histidine (12) and arginine (13). In other cases, a few kinds of unknown peptides were found. In every case, qualitatively and quantitatively a remarkable increase of amino acids and peptides was noted more than in other dieseases.

Discussion

Tiba[3] and Yosizawa[4] proved that hexosamine in gastric juice is largely glucosamine, and only partially chondrosamine.

But it was not obvious whether or not hexosamines are present in gastric juice in free form. Contrary to Cagianut, we could not recognize free hexosamines in gastric juice in the present experiment. These authors indicated the presence of cystine or cysteic acid, and we also found them.

The nitrogen values suggested that amino acids and polypeptides in gastric juice increase in gastric cancer much more than in othere diseases.

This was ascertained by the results of paper partition chromatography. Especially it must be of significance that glutamic acid, glycine, proline, tyrosine, etc., were found only in the case of gastric cancer among other diseases.

Summary

1. We divided gastric juice of patients with gastric lesions into 5 groups, and compared total and free nitrogen of non-protein nitrogenous compounds.

2. Paper partition chromatography showed the presence of free amino acids in gastric juice of all the groups of patients, but in gastric

cancer, the amount and number of amino acids surpassed those in other diseases.

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Chédiak and Higashi's Disease*. Probable Identity of "A New Leucocytal Anomaly (Chédiak)" and "Congenital Gigantism of Peroxidase Granules (Higashi)"

By

Akira Sato**
(佐藤　彰)

From the Department of Pediatrics, Tohoku University, Sendai

(Received for publication, February 12, 1954)

In the present paper I desire to describe a new blood disease, preliminarily reported a few months ago.[1] How I have come to describe the present blood dyscrasis is rather peculiar as well as accidental.

In Sept. 1953, O. Higashi published a new clinical entity for which he suggested the name of "Congenital Gigantism of Peroxidase Granules".[2] The details of it I refer to his original paper, but I shall relate how he came to find out this new clinical entity.

What surprised him was the peroxidase picture of blood of that case of his. He usually would not use the Giemsa or the Wright stain first, but used to apply the Tohoku Pediatric Method (a modification of Sato and Sekiya's peroxidase reaction)[3-4] on blood smears in every case, because, by that method, he could, however badly a blood film might be prepared, differentiate lymphocytes correctly from (peroxidase-positive) monocytes. He had been using it since 1944. So when he saw in 1950 the peculiar case of his own, he was surprised at the peroxidase picture his case had presented. Abnormity was not the word for it. It was a *monstrous peroxidase picture*, as will be shown below.

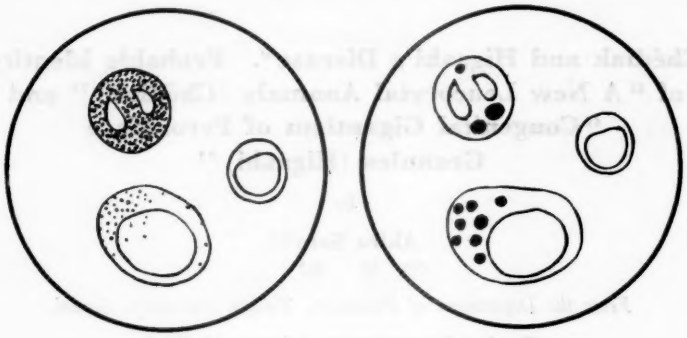
Every hematologist with some experience with the peroxidase reaction thinks, I believe, that the peroxidase picture which the neutrophil, a myeloic element, presents is not only general, but even universal (Cf. Fig. 1, a).

In this particular case of his own, he found that neutrophils were peroxidase-stained like the cell (Fig. 1, b), in wihch those small peroxidase granules that would normally have ocurred diffusely scattered all over the cytoplasm did gather in a few or several spots of it to form round clumps or

* Read at the Monthly Meeting of the Tohoku Medical Society, held 21 May, 1954.

** Professor emeritus.

Fig. 1. Peroxidase reaction (Upper: neutrophile. Lower: monocyte. Cell without granules: lymphocyte), diagrammatically drawn.



a. Normal b. Higashi's disease

globes leaving only a few granules scattered in the interspace and in surrounding parts. And attention must be called to the fact that such a monstrous peroxidase picture (Fig. 1, b) was manifested by almost all the *neutrophils* of blood.

Such a gigantism of peroxidase granules is characteristic, not only of the neutrophil, but also of the monocyte. The fact that this gigantism is characteristic of the leucocytes of the myeloic system was still better known when aspirated bone marrow films were peroxidase stained (see below).

Besides the neutrophil and the monocyte, as marrow punctates show, the eosinophil, the myelocyte, eosinophilic as well as neutrophil, and the myeloblast show the giganticism of peroxidase granules. Lymphocytes and megakaryocytes were peroxidase-negative, as had been expected.

On the basis of the above described anomaly of peroxidase granules that affected the entire myeloic system, a new clinical entity was suspected. Now, the mother of the present case was, as she stated, afraid that the child (the present case) might die of the present illness,—rather she *prophesied his death*, because she had, as she stated, already experienced the death of three children who had succumbed to a similar affection. Why this precise prophecy? Because these three children had, as she stated, in common with the present case, albinism with photophobia (with pale color of hair), pigmentation of uncovered parts of skin, frequent occurrence of pustules with generalized lymphadenopathy and marked abdominal distention (due to hepatosplenomomegaly), whereas, as she added, the other three children without these symptoms were well and healthy.

As the present case had xeroderma pigmentosum as one of his diseases or symptoms, it was at first thought that this abnormal pigmentation might

have something to do with the gigantism of peroxidase granules. Higashi then had the opportunity of examining three cases of xeroderma, two cases of retinitis pigmentosa and one case of total albinism, but he found no abnormity of the peroxidase granules of leucocytes in any of these cases.

Though he was thus unable to find the cause for which this monstrosity of peroxidase would occur, yet it seemed highly probable that the present case and his sibs had an inborn error of leucopoiesis in common. And he suggested for this new clinical entitity the name: " Congenital Gigantism of Peroxidase Granules ".

Long, long ago I devised in collaboration with Sekiya a peroxidase reaction.[3] Though I published it in English in 1926. I had been using it since 1920 (up to 1948) on every patient from whom blood was obtained. I have never seen such a peroxidase picture as was presented in this particular case of Higashi's. It is not an abnormity that only a few cells of the same kind present, but one affecting all the cells of the myeloic system.

Such a monstrosity of peroxidase picture was for the first time published. I am quite sure of it. Our peroxidase reaction seems to be fairly widely used under Sato and Sekiya's peroxidase stain[3] in the American continent and under Sato's peroxidase reaction[4] in the European continent. But such a case as the present one has not within my knowledge been published. It is highly probable that Higashi's case is the very first one of this peculiar monstrosity of peroxidase picture.

New Leucocytic Anomaly of Constitutional and Familial Character

Higashi's case was reported in the last autumn in the 51st Meeting of the Miyagi Prefectural Pediatric Society. Soon after it, I came across Moises Chédiak's article.[5] His Fig. 3, which showed Giemsa-stained leucocytes of sternal puncture, attracted my attention first, because in the cytoplasm of some leucocytes in that figure more or less large vacuoles with a large enclosed body, were represented. Then I saw the pictures of the children on the next page and read the word " photophobia ", then saw his conclusions, the digest of which will be something like the following :—' This is a familial anomaly of panleucopoiesis affecting granulocytes, monocytes and lymphocytes. The Giemsa-stained film shows that profound changes and inclusions in the cytoplasm, anomalies of granulations and of nuclear structure occur in these cells. The affected children are albinic or " blond pâle " and photophobic, and show a normal nutritional, somatic and functional development up to 6 years of age, when they succumb to intercurrent infection. Sulfonamides are ineffective.'

In his case *peroxidase was not examined*, so that of course any feature of

the peroxidase reaction cannot be conceived, but there exists a great similarity between his and Higashi's cases, as will be related below.

Comparison between Higashi's and Chédiak's Anomaly

Chédiak's anomaly (reported from Habana)	Higashi's anomaly (reported from Sendai)
A. Familial leucocytal anomaly	A. Familial leucocytal anomaly
B. Patient:—albinic infant with photophobia; non-albinic sibs healthy	B. Patient: albinic infant with photophobia; non-albinic with healthy
C. Fatality: death before 7 years of age	C. Fatality:—death before 3 years of age

These three items of comparison will be discussed below:—

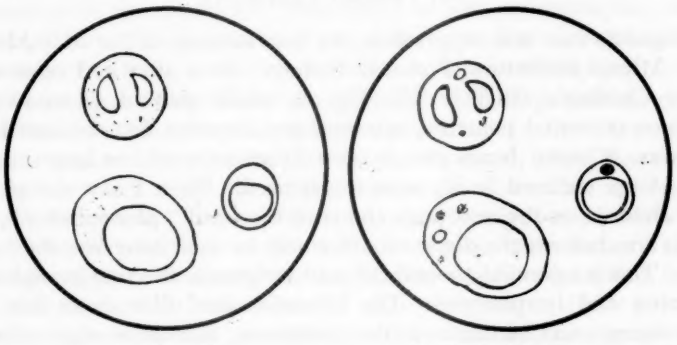
A. Familial leucocytal anomaly

It is evident that the anomaly is familial from both Chédiak's description and the pedigree in Higashi's paper. Consaguinity is common.

The Giemsa stained neutrophils show esp. in the cytoplasm an abnormal arrangement of neutrophil granules; in a word, granules form several clumps instead of being scattered all over the cytoplasm.

The description of the lymphocyte differs according to Chédiak and Higashi. According to the former, in nearly every lymphocyte (Fig. 2) an inclusion body, distinctly stained, is seen, while in Higashi's case such occurred in only 20% of lymphocytes. But the abnormal change that occurred in lymphocytes seems to be the same, so that the difference between Chédiak's and Higashi's cases is only a quantitative one.

Fig. 2. Giemsa stain (Upper: neutrophile. Lower: monocyte. Cell without granulations: lymphocyte), diagrammatically drawn.



a. Normal b. Higashi's disease

The change of the neutrophil seems to be almost the same, as far as the Giemsa stained cytoplam is concerned. The chromatin is abnormal according to Chédiak, while Higashi states that it is now thin, then thick. There is probably no essential difference.

The Giemsa-stained monocytes are probably equal in both cases (Fig. 2).

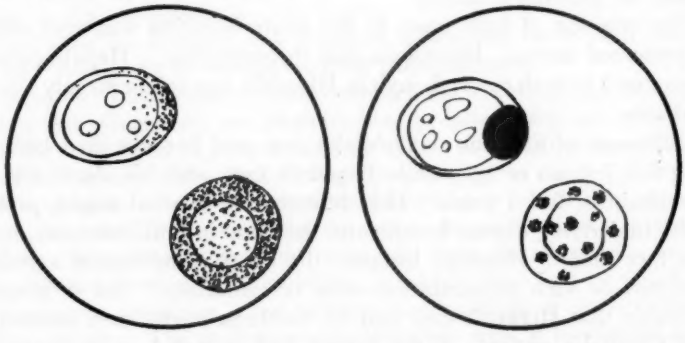
As to the eosinophil leucocyte (in the peripheral blood), there is no description in Higashi's paper, probably because the infant was seen only on the occasion of fatal infection, when all or almost all peripheral eosinophils must have disappeared. So I shall discuss this kind of cells in the following :—

Leucocytes in the marrow punctate :—

a. Eosinophiles: The peripheral eosinophil presents according to Chédiak a very particular aspect showing two types of characteristically large granulations: one type is spheric and unequal, and the othe ellipitic reminding us of horse eosinophil. Higashi describes the marrow eosinophils: The specific granules are large and different in size, and they are mostly spherical, but some of them appear rather polymorphous, presumably showing a coalescence of two or three original less large spherical granules.

b. In Higashi's case (Cf. Fig. 3) marked vacuolization occurs often in myelocytes and myeloblasts, esp. in the latter, in which extraordinally large round vacuoles, each with a round large granule or aggregate of granules, as are seen in Fig. 3. of Chédiak's paper. Inclusion bodies are

Fig. 3. Peroxidase reaction of marrow cells (Upper: myeloblast. Lower: myelocyte), diagrammatically drawn.



a. Normal b. Higashi's disease

frequently seen in Higashi's case as well as in Chédiak's.

c) Other kinds of cells :—

In Higashi's case neither erythroblasts nor megakaryocytes show particular abnormity, while both of them show pathologic regeneration.

In his paper Chédiak states: " One finds nothing abnormal in size, nucleus and cytoplasm of all the elements of the megakaryopoietic system ",

further " All the elements of the erythropoietic series are absolutely normal."

B. Patients

Both Chédiak's and Higashi's cases are albinic infants with photophobia; they were both probably born with these symptoms. But in other respects these cases differ from each other.

Chédiak's case seems to have developed normally with only these two symptoms, but Higashi's case began to show dark pigmentation on uncovered parts of the skin and a more or less darker pigmentation of albinic hair about five months of age. His growth and development were rather retarded. With an expectation that pigmentary abnormity might have something to do with abnormal blood cells, as has been stated already, Higashi examined three cases of xeroderma pigmentosa, two cases of reitnitis pigmentosa and one case of total albinism, but no such abnormity was found.

And it is not excluded that a retardiation of growth and development in Higashi's case would have been be due esp. to avitaminosis D: it will not be difficult to suppose that his mother had been keeping him rather in the dark on account of his photophobia, esp. as skin pigmentation began, as she stated, to occur from the time he was exposed to the sun. It is probable that present blood dyscrasia could occur without such complication as xeroderma pigmentosa.

It is to be emphasized that in both families the sibs not born with albinism are growing normally.

The reaction of both cases to the acute infection was very similar; they presented anemia, leucopenia and thrombopenia. Hepatosplenomegaly occurred in both cases, though in Higashi's case splenomegaly was very remarkable.

Difference of life span: Chédiak's case and her sibs died before attaining full 7 years of age, while Higashi's case and his dead sibs died before attaining full 3 years. This difference of survival might, provided that the underlying disease be one and the same in both instances, be due to the fact that in Higashi's instance the other complication xeroderma pigmentosa or such pigmentation—was concomittant. But it is equally conceivable that Higashi's case and his brethren might have attained the age of Chédiak's patients, if the former had been fed as the latter. In rural villages in our country, nutritional state of affairs is often very poor, esp. in the age of weanling. Higashi's own case—the infant reported in his paper—was esp. shortlived. It is not to be excluded that Higashi's case and his brethren could have attained the age of the cases reported in Chédiak's paper.

C. Fatality

The mother of Chédiak's case prophesied that her child of 6 years

would die of the present affection, because she had already experienced a similar death of her three other children with albinism and photophobia. The mother of Higashi's case prophesied that her infant of 11 months would die of the present affection, because she had already experienced a similar death of her three other infants with albinism, photophobia and pigmentation.

Both cases presented the syndrome of an acute infection, but Chédiak's case did not respond to sulfonamides, blood transfusion etc, though he could not try penicillin, because this antibiotic had not been put on the market yet. In Higashi's case penicillin was tried, but in vain. It is not difficult to suppose that this antibiotic might have been inefficient in Chédiak's case too.

It is highly probable that Chédiak's and Higashi's diseases are only two variations of one and the same disease.

Chédiak and Higashi's Disease

The following is in no way a text book like description of the disease. It is written here for quick orientation and esp. in order that, in case one should come across a similar case, no data important might remain omitted or unexamined.

Etiology. It is a familial and probably congenital disease and affects children of both sexes. The disease is probably transmitted through apparently healthy persons. Consangunity is probably one of the most important factors. The heredity is not sex-linked.

In tracing the pedigree, it is of utmost importance not to omit those who died very young and the symptoms these had manifested.

Symptoms. Infants and children who are the victims of this disease have albinism with albinic hair and photophobia, the latter being noticed soon after birth. But they seem to show a fairly or almost normal growth and development, until they succumb to an infection, from which there can be no recovering.

The question is whether or not the pigmentation which may occur after birth on uncovered parts of albinic skin and in the albinic hair is a facultative symptom. Special attention ought to be paid upon the albinic skin on which some pigmentary anomaly or darkneing of albinic hair may be found. The most important symptoms is the pathognomonic change of blood elements.

Blood picture: The pathognomonic change is seen, if peroxidase reaction is applied to air dried blood films. Instead of the usual peroxidase picture of leucocytes of the myeloic system, a monstrous feature (Cf. Fig. 1) is presented. This feature can be diagnostic by itself.

If the Giemsa stain is applied, some or all lymphocytes are seen to contain each one relatively large round dark stained inclusion body (Fig. 2, b) in the cytoplasm, and a myeloblast in the marrow punctate shows a large vacuole (or vacuole-like structure) with an inclusion body (Fig. 2).

Symptoms of acute infection. If the patient is attacked by acute infection,

he will react with the following symptoms: fever, infection of upper respiratory tract, anemia, leucopenia, thrombopenia, prolonged bleeding time, hepatosplenomegaly, lymphadenopathy and possibly jaundice.

Hepatosplenomegaly:—

The spleen was very large in Higashi's case. As the family of this case live in the country, it was not excluded that they esp. infants were, as is quite often the case in our country, undernourished, so that they used to develop pustules due to which—or due to such recurrent infection—the spleen (and possibly the liver, therefore the abdomen) may have been gradually swollen already before the fatal infection ensued.

Diagnosis: The diagnostic trias may be:—

1) Albinism with photophobia, 2) early death of albinic brethren and 3) gigantism of peroxidase granules of myeloic leucocytes.

In such a case, when no acute infection exists, special attention ought to be paid upon any existence of any pigmentation upon albinic skin or hair, and further upon lymphadenopathy and possibly upon splenohepatomegaly. Ask the mother if the child has had any pustular eruption occasionally.

Prognosis: However healthy—except for photophobia—the albinic in fant or child may appear, death due to acute infection will be inevitable in the 7th year of life at the latest.

Prophylaxis. As the patient will succumb quite easily to acute infection, it may possibly be the only one measure of life-prolonging to keep him from infection.

Treatment: Neither sulfonamides nor penicillin seem to be capable of saving the patient from the fatal infection. The other antibiotics may be tried, but it is a question whether or not these will take effect.

Remarks

1. The name: "Congenital Gigantism of Peroxidase Granules":—

May we use "Congenital Gigantism of *Oxidase* Granules" instead? I was very curious to know the result of oxidase reaction (of Winkler and Schulze), because—perhaps against the expectation of some (or many?) hematologists, I did not expect necessarily that the oxidase reaction would occur in the same way morphologically. (I will explain the "why not?" in another paper of mine—The Quantitative and Qualitative Anomaly of Peroxidase Reaction).

In the actual experiment of Higashi's, however, the oxidase reaction came out exactly as the peroxidase reaction. Then could not the present disease also be called "Congenital Gigantism of Oxidase Granules"? Yes, *it could in all probability.* But Higashi had not been using the oxidase reaction in routine work, while he had been using the peroxidase reaction daily. So it is much safer to use "Congenital Gigantism of Peroxidase Granules" alone for the time being.

2. I desire to ask those interested in hematology to examine blood in

every albinic individium, adult or child, and to ask them, even if myeloic leucocytes on the first few films would not present any peroxidase abnormity, to examine many more to find if there are some cells showing an inclination of the abnormity, further to try *a marrow puncture* to see whether some marrow cells are abnormal or not.

Here for convenience' sake, I shall briefly describe our peroxidase reaction.

Reagents: Solution A. 0.5% copper sulfate ($CuSO_4 \cdot 5OH_2$) solution
Solution B. Benzidine—peroxidase solution.

Rub 0.2 g. benzidine in a mortar with a few drops of water. Add 200 cc. of water and shake well (Filtration is not always necessary.) Then add 4 drops of 3% H_2O_2 (this should be fresh) to it.

Solution B will last a long time if kept in the dark. (It is recommendable to try whether or not Solution B has been well prepared—as is described in Wintrobe's Clinical Hematology, " It may be tested by mixing Solutions A and B in a test tube. If this mixture does not become blue, the reagents are at fault ".

Solution C (Counter strain). 1% aqueous Safranin-O solution.

Technic: Apply Solution A to an air-dried blood film for a short time (10–40 seconds may be sufficient). Then pour off most of it and apply Solution B for two minutes, then wash with water. And apply Solution C for 1–2 minutes.

If all the solutions are well prepared and the technic is right, then the very first trial will succeed in presenting a beautiful peroxidase picture.

3. Fatality and abnormal leucopoiesis:—

Though it is much too premature to discuss the relation between the fatality and the abnormal leucopoiesis in the present disease, it seems to me that the former is closely related to the latter, so much the more, as the patient invariably succumbs to acute infection.

It is also to be considered that there may be a definite relation between the abnormal leucopoiesis in question and the brief span of life.

4. Though this is a mere speculation, it is not excluded that in Chédiak's case the gigantism of peroxidase granules may not be so much pronounced as in Higashi's case, or that in the former case not all the granulocytes show such a gigantism of peroxidase granules, just as in Chédiak's case all lymphocytes showed each an inclusion body, while in Higashi's case only a minority of the cells showed this abnormity. This might be thought of in connection with a longer span of life in Chédiak's disease.

5. Whether or not the red system is utterly normal remains to be solved.

Summary

Higashi published " Congenital Gigantism of Peroxidase Granules " in Sept. 1953, in which I was very much interested, because I had not seen any case of such an abnormity during my own 30 years' experience with the peroxidase reaction.

Then I happened to read an article published in 1952 by Chédiak: Nouvelle anomalie leucocytaire de caractère constitutionnel et familial.

On perusal, I came to consider both diseases as two different manifestations of one and the same clinical entity, though there was no description of peroxidase or oxidase raaction in Chédiak's paper. So I desire to suggest for this new clinical entity the name: Chédiak and Higashi's Disease or Chédiak-Higashi's Leuco-anomaly.

Conclusions

1. The name of " Chédiak and Higashi's Disease " or " Chédiak-Higashi's Leuco-anomaly " is suggested for both " Chédiak's " Nouvelle anomalie leucocytaire de caractère constitutionnel et familial " and Higashi's " Congenital Gigantism of Peroxidase Granules ".

2. The diagnostic trias may be: 1) Albinism with photophobia, 2) early death of albinic sibs and 3) very characteristic (even pathognomonic) blood morphology.

3. The patient seems to enjoy an apparently uneventful growth and development (on a rational regimen) up to a certain age (1–6 years of age), when he or she succumbs *invariably* to acute infection, sulfonamides or penicillin being of no effect.

4. The early death seems to be related to a leucocyte anomaly which is, it seems, transmitted by (either or both of) healthy consanguinous parents to some of their children.

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Pharmacological Studies on Proteins from Carcinomatous and Non-carcinomatous Tissues and Body Fluids

By

Hirosi Tiba

(千 葉 博)

From the Medical Clinic of Prof. T. Kurokawa, Tohoku University, Sendai

(Received for publication, February 12, 1954)

Years ago, Grave and Römer[1] prepared a hemolytic substance from gastric juice of patients with gastric cancer.

Kimura[2] separated from uterine cancers a toxic substance that will cause the decrease of blood pressure of rabbit and have the poisonous effect on a frog heart. Recently, Iwatsuru and others[3] pointed out the appearance of a peptide (KIK-factor) in the secretion from cancer stomach. Nakahara and Fukuoka[4] isolated a thermostable non-heat coagulable, water soluble and alcohol precipitable substance from the cancer tissues that reduced the liver catalase activity. The present writer separated water soluble proteins from carcinomatous and non-carcinomatous tissues and body fluids. The preparations and pharmacological observations of these materials was processed as follows:

Experimental

(A) Preparation Procedure

1) Preparation of the protein from the tissue of gastric cancer (C-P). Proper tumor tissue was dissected from extirpated gastric cancers, and after removing off blood and necrotic matters by washing, weighed and cut into small pieces.

(i) Extraction: 200 g. of the tissue which were sufficiently masticated were agitated with 5 volumes of water for 5 days (room temperature 14–17°C), using toluene as a preservative, and centrifuged. The residue was treated likewise twice. The centrifuged supernatant liquids were fractioned.

(ii) Treatment with baryta: To the extract was added the saturated $Ba(OH)_2$ (the pH was schifted to 9.0), stood for 2 hours and centrifuged.

(iii) The treatment with acetic acid: the supernatant liquid was acidified with 30% acetic acid to pH 4.2 with addition of about 0.1 g.

NaCl per 100 cc. of the fluid and the flocculent precipitates were centri fuged off. The supernatant liquid was treated likewise with baryta and acetic acid twice. And barium was eliminated with just sufficient quantity of sulfuric acid (1 *N*).

(iv) Dialysis: The supernatant liquid was distilled under diminished pressure to about 200 cc. and dialyzed through cellophane against running water for 4 days.

(v) Concentration and preparation with alcohol: It was freed from some precipitation and distilled under diminished pressure to about half volume and further condensed to a syrup after centrifuging off the precipitate. Sufficient alcohol was added and precipitate that appeared was collected and washed with absolute alcohol and dried.

(vi) Purification: It was dissolved in water and acidified by means of 10% acetic acid to pH 4.2. Insoluble part of it was centrifuged off and electrodialyzed (a Pauli apparatus with cellophane diaphragms, 150 volts) for 24 hours. After centrifuged off the precipitate newly appeared, supernatant liquid in the middle compartment was distilled to a syrup, precipitated with alcohol and dried. Yield: about 160 mg.

2) Preparation of the protein from normal gastric mucosa (M-P). Water soluble protein from human gastric mucosa was prepared in similar manner as mentioned above.

Qualitative Assays. The results are as follows:

	C-P	M-P
Biuret	##	##
Molisch	++	++
Phosphorus	+	+
Neuberg-Saneyoshi	+	+
Hydrolysable sulfur	±	±

±: faintly positive, +: slightly positive, ++: markedly positive, ##: strongly positive.

Quantitative Analysis

The analytical figures obtained are given in Table I.

3) Preparation of the protein from gastric juice of gastric cancer (CJ-P).

Human gastric juice was collected from patients with gastric cancer. The diluted juice was percolated through a gauze and then made clear by filtration through a creased paper. The filtrate was evaporated to a syrupy density under reduced pressure and 95% alcohol was added. The precipitates were washed with absolute alcohol and dried. 1.7 g. of the powder was dissolved in 50 cc. of water and centrifuged repeatedly to get a transparent supernatant liquid. The supernatant liquid was acidified by means of 30% acetic acid to pH 4.2, centrifuged and dialyzed against

TABLE I

Composition of the Proteins from the Tissue of Gastric Cancer and Normal Gastric Mucosa

	In per cent	
	C-P	M-P
Nitrogen*	12	12.4
Hexosamine†	9.1	9.4
Non-Amino Sugar‡		
Galactose	7.6	7.3
Mannose	2.7	2.3
L-fucose	4.7	4.6
Ash	3.2	2.8

* Micro Kjeldahl. † Blix method. ‡ Masamune and Ogawa method.

running water for 2 days. pH of the solution rose thereby to 5.4–5.6.; It was then freed from some precipitate, distilled under diminished pressure to a syrupy consistency and 95% alcohol was added. The precipitates were washed with absolute alcohol and dried. (300 mg.) It was dissolved in 20 cc. of water, separated from the insoluble part and acidified by 10% acetic acid to pH 4.2. The clear supernatant liquid was electrodialyzed for 24 hours. The some precipitates that had appeared were centrifuged. The final supernatant liquid was distilled to a syrup. Sufficient volume of absolute alcohol was added to precipitate all the insoluble part which was collected and washed with absolute alcohol and dried. Yield: 80 mg.

4) Preparation of the proteins from non-carcinomatous gastric juice.

Water soluble proteins from gastric juice of patients with chronic gastritis (GJ-P), gastric ulcer (UJ-P), and duodenal ulcer (DUJ-P) were prepared in a similar manner as above.

In the present experiment, quantitative analysis of the proteins from carcinomatous and non-carcinomatous gastric juice was not accomplished, though they exhibited remarkable peculiarities in serological activity as will be described below.

Table II illustrates the specific but low potency of the cancer protein (B-group).

Table III shows that two proteins from carcinomatous and non-carcinomatous gastric juice (O-group) inhibit the agglutination of O-erythrocytes by virtue of the anti-E eel serum and that the former has low potency to prevent erythrocytes from agglutination by virtue of the anti-E serum.

5) Preparation of the protein from the tissue of hepatoma (CL-P). Tumor tissue was dissected from hepatoma that was supplied from the In-

TABLE II

Agglutination of Erythrocytes by Normal Anti-sera*, treated with Proteins of Carcinomatous and Non-carcinomatous Gastric Juice (B-Group)

Group of protein	Group of erythro-cytes	Dilution of substance								
		1% (original)	×10	×10²	×10³	×10³ ×2	×10³ ×4	×10³ ×8	×10³ ×16	×10³ ×32
B (gastric cancer)	A	±	+	++	###	###	###	###	###	###
	B	−	−	−	+	+	++	###	###	###
B (chronic gastritis)	A	±	+	++	###	###	###	###	###	###
	B	−	−	−	−	−	−	+	++	###

* A and B sera had agglutinin titers 160 and 480 respectively.

TABLE III

Agglutination of O-Erythrocytes by Anti-E Eel Serum.† treated with Proteins of Carcinomatous and Non-carcinomatous Gastric Juice (O-Group)

Anti-E sera had agglutinin titer 1280

Group of protein	Group of erythro-cytes	Dilution of substance										
		1% (original)	×2	×4	×8	×16	×32	×64	× 128	× 256	× 512	× 1024	× 2048
O (gastric Cancer)	O	−	−	−	−	−	−	−	−	+	++	++	++
O (Chronic Gastritis)	O	−	−	−	−	−	−	−	−	−	−	++	++

† Kindly supplied by the Medico-chemical Institute of Prof. H. Masamune.

stitute of Pathology (Prof. S. Nasu) where the autopsy had been made. And preparation of the protein from the tissue of hepatoma was made in the similar way as in the case of gastric cancer.

6) Preparation of the proteins from carcinomatous and non-carcinomatous ascitic fluids (CA-P, NCA-P).

Water soluble proteins from carcinomatous and non-carcinomatous ascitic fluids were separately prepared according to the procedure of Tiba *et al.*[5] at the Medico-chemical Institute, where Prof. H. Masamune is the Director.

Protein from carcinomatous ascitic fluid (CA-P) that originated in stomach cancer and that of non-carcinomatous ascitic fluid (NCA-P) due to liver cirrhosis resembled each other in qualitative as well as in quantitative aspect but differed in serological and immunological point of views.

7) Preparation of the protein from placenta (P-P). A batch of water soluble fraction of placenta which gave the cancer skin-reaction was separated and purified by Oh-Uti *et al.*[6] and Ogawa[7].

Fresh placentas that had been freed from amnion and decidua were each cut into pieces, washed with water and shaken with 10 volume of water for 3 days. The percolate was centrifuged. The tissue residue was subjected to a similar extraction twice. The supernatant fluids were distilled to about 1/5 volume, and were added with the saturated baryta to a pH of 8.8–9.0. The centrifuged-supernatant was acidified with 30% acetic acid to a pH of 4.2–4.4 and centrifuged. The yellowish clear mother fluid was dialyzed against running water until no barium remained. Some precipitate was filtered off. The filtrate was distilled in vacuo to a syrup, precipitated with alcohol and dried.

The products were dissolved in water and the insoluble part was discarded. Barium was taken off with just sufficient sulfuric acid (1 *N*). The transparent brownish solution was dialyzed against running water for 3 days to take off remaining barium completely. It was distilled, precipitated with absolute alcohol and dried.

Biuret, Molish and phosphorus tests were all positive.

(B) Effects of Proteins on the Blood Pressure and the Respiration of Rabbit

A rabbit was anesthetized with urethane in doses of 1 g. per kg. of body weight, and when the animal was completely under the influence of the anesthetic, an incision was performed. The carotid artery was exposed on one side and a cannula was inserted which was connected by means of a rubber tube with the manometer. And tracheacannula was inserted into trachea by tracheotomy and connected with tambour (Marey). These were arranged for tracig. The following materials were administered to the ear-vein at the rate of 10 mg. per every g. of weight.

Results

By administration of C-P and CJ-P, the blood pressure gradually fell down as seen in Figs. 1 and 3, but other proteins-M-P, UJ-P, GJ-P, CL-P, CA-P, NCA-P and P-P did not have any effect on the blood pressure as seen in Figs. 2, 4, 5, 6, 7, 8 and 9. On the contrary, the respiratory recording was not influenced by all proteins.

Judging from the results mentioned above only proteins from the tissue and juice of gastric cancer show any effect on the blood pressure of rabbits.

(C) Effect on the Excised Frog Heart

The writer of observations on the effect on the excised frog (Rana

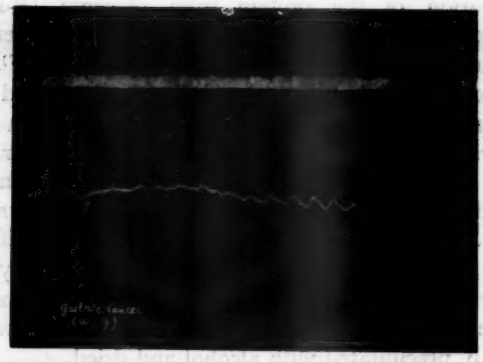

Fig. 1. The sample of the respiratory (the upper trace) and the blood pressure (the under trace) tracings.
The protein of gastric cancer was injected intravenously in a dose of 10 mg. per kg. body weight of rabbit.

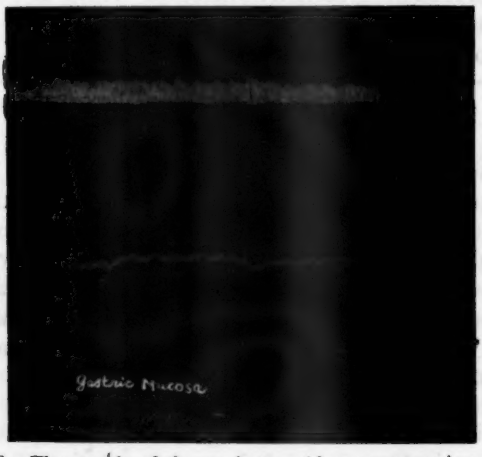

Fig. 2. The sample of the respiratory (the upper trace) and blood pressure (the under trace) tracings.
The protein of nomal gastric mucosa was injected intravenously in a dose of 10 mg. per kg. body weight of rabbit.

nigromaculata) heat by the Yagi-Straub method.

Results

1) C-P

With 0.001% concentration, no noticeable effect was seen, but with 0.01% concentration, the amplitude of heart beat lowered a little. And with the 0.1% solution, it became remarkably lower, but the frequency

Fig. 3. The sample of the respiratory (the upper trace) and the blood pressure (the under trace) tracings.
The protein of carcinomatous gastric juice was injected intravenously in a dose of 10 mg. per kg. body weight of rabbit.

Fig. 4. The sample of the respiratory (the upper trace) and the blood pressure (the under trace) tracings.
The protein of gastric juice of gastric ulcer was injected intravenously in a dose of 10 mg. per kg. body weight of rabbit.

of heart beat did not decrease (Cf. Fig. 10) and with 1% concentration, the beat stopped in a few minutes.

2) C-P (treated with hot water)

C-P was dissolved in water and heated in a boiling water bath at 100°C for 30 minutes.

The results were the same as in C-P. With 0.1% concentration, the amplitude of heart beat became especially lower. (Cf. Fig. 11).

3) M-P

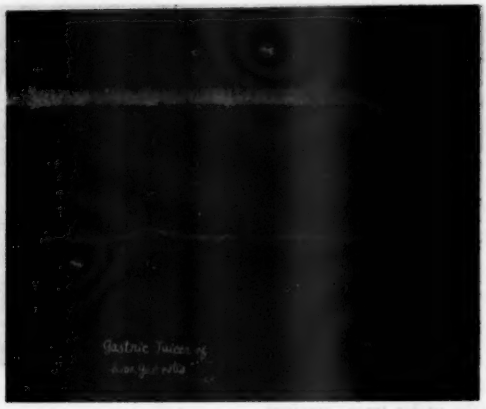

Fig. 5. The sample of the respiratory (the upper trace) and the blood pressure (the under trace) tracings.
The protein of gastric juice of chronic gastritis was injected intravenously in a dose of 10 mg. per kg. of body weight of rabbit.

Fig. 6. The sample of the respiratory (the upper trace) and the blood pressure (the under trace) tracings.
The protein of hepatoma was injected intravenously in a does of 10 mg. per kg. body weight of rabbit.

With 0.001–0.01% concentration, noticeable effect was not seen. With 0.1% concentration, the amplitude of heart beat moderately lowered (Cf. Fig. 12), and with 1% concentration, the beat of heart stopped.

4) CJ-P

With the 0.001% solution, the tension, the amplitude and the frequency of heart were not disturbed, but with the 0.01% solution, they rose slightly. With 0.1%, the amplitude lowered at first and the tension rose temporarily, and then the amplitude increased. (Cf. Fig. 13).

Fig. 7. The sample of the respiratory (the upper tracing) and the blood pressure (the under trace) tracings. The protein of carcinomatous ascitic fluid was injected intravenously in a dose of 10 mg. per kg. body weight of rabbit.

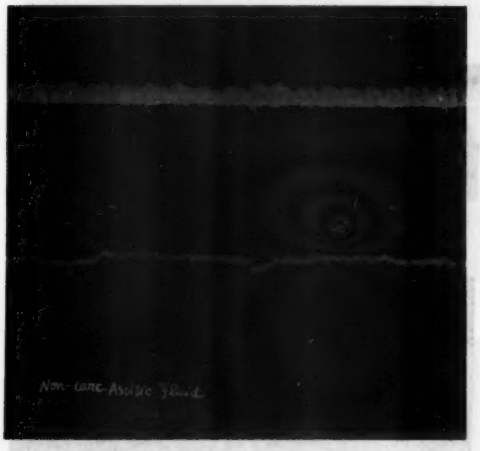

Fig. 8. The sample of the respiratory (the upper trace) and the blood pressure (the under trace) tracings. The protein of non-carcinomatous asctic fluid was injected intravenously in a dose of 10 g. per kg. body weight of rabbit.

With the concentration of 1%, the beat of heart became asymmetric and finally stopped at the dilated position.

5) UJ-P

With 0.001–0.1%, no change was observed. With the 0.1% solution, the temporary increase of tension and a decrease in amplitude were observed. (Cf. Fig. 14).

6) GJ-P

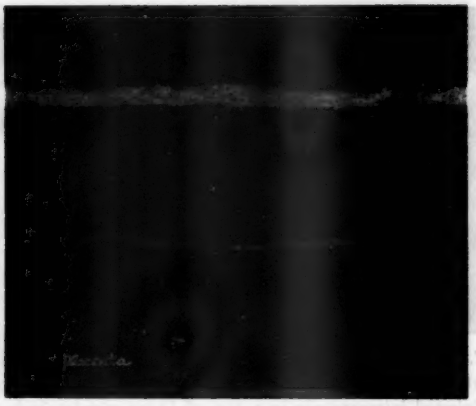

Fig. 9. The sample of the respiratory (the upper trace) and the blood pressure (the under trace) tracings. The protein of placenta was injected intravenously in a dose of 10 mg. per kg. body weight of rabbit.

Fig. 10. With the protein of gastric cancer, 0.1%.

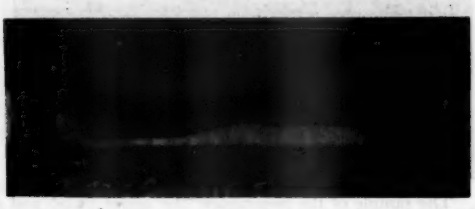

Fig. 11. With the protein of gastric cancer, treated with hot water, 0.1%.

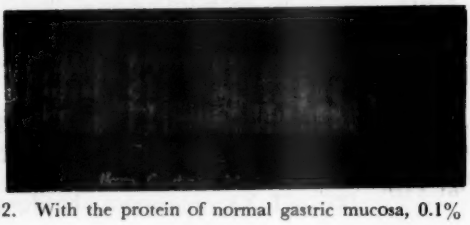

Fig. 12. With the protein of normal gastric mucosa, 0.1%

Fig. 13. With the protein of carcinomatous gastric juice, 0.1%.

Fig. 14. With the protein of gastric juice of gastric ulcer, 0.1%.

Fig. 15. With the protein of gastric juice of chronic gastritis, 0.1%

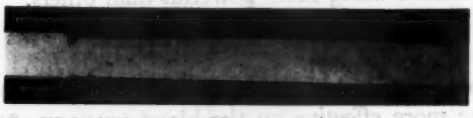

Fig. 16. With the protein of hepatoma, 0.1%.

Fig. 17. With the protein of carcinomatous ascitic fluid, 1%.

Fig. 18. With the protein of non-carcinomatous ascitic fluid, 1%.

With 0.001%, a noticeable disturbance was not seen, and at 0.01–0.1%, the amplitude increased a little (Cf. Fig. 15), with the 1% solution, the

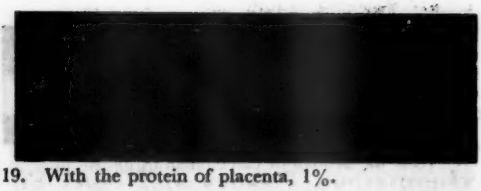

Fig. 19. With the protein of placenta, 1%.

beat of heart stopped temporarily and then the systolic action became strong remarkably.

7) CL-P

With the 0.1% solution, the amplitude decreased for a time (Cf. Fig. 16). At other concentrations, the same tendency was seen as in GJ-P.

8) CA-P

9) NCA-P

10) P-P

As to CA-P (Cf. Fig. 17), NCA-P (Cf. Fig. 18) and P-P (Cf. Fig. 19), a little increase in amplitude was observed with the 1% solution.

However, there were no perceptible changes with other concentrations.

From the results mentioned above it is obvious that these 10 materials have some effect on the heart function, especially proteins of the tissue and juice of gastric cancer being more powerful than others.

Summary and Conclusion

1. The proteins in the tissue of gastric cancer and carcinomatous gastric juice are more effective on the blood pressure of rabbits and the function of the isolated frog heart than the corresponding proteins from normal gastric mucosa and non-carcinomatous gastric juice.

2. The proteins in carcinomatous and non-carcinomatous ascitic fluids and human placenta are not poisonous.

3. The protein in carcinomatous gastric juice is less active in the group-specific reaction than the corresponding proteins from non-carcinomatous gastric juice.

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On Paper Partition Chromatography of Proteins and Carbohydrates from Carcinomatous and Non-carcinomatous Tissues and Body Fluids

By

Hirosi Tiba and **Sumio Isikawa**
(千 葉 博) (石 川 澄 雄)

From the Medical Clinic of Prof. T. Kurokawa, Tohoku University, Sendai

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Investigations on the group carbohydrates from gastric cancer and gastric mucosa were reported by Masamune and his cooperators[1)2)3)].

Tiba[4)] has prepared proteins which are active pharmacologically and serologically from the carcinomatous and non-carcinomatous tissues and body fluids.

To identify sugar and amino acid components in these materials, the writers made use of paper partition chromatography.

Experimental

I. Experiments regarding the Sugar Components

Technique

Paper partition chromatography of sugar was attempted this time in the descending way which served the purpose more than in the ascending.

Then the chromatography proceeded according to Masamune and Yosizawa.[5)] Toyo filter paper No. 2 is cut into strips of 60 cm. × 5 cm. for standard and the hydrolysate of materials.

Sampling

Of a solution, containing sugars in concentration of 2% with respect to each individual sugar and acidulated to pH 5.8 with a dilute H_2SO_4 (0.1 *N.*), was pipetted 0.013–0.015 cc. on a paper strip at a distance of 10 cm. from the upper edge. Sugars to be used were glucosamine hydrochloride (crystalline), galactose (E. Merck), mannose (Tanabe) and rhamnose (Tanabe).

About 30–40 cc. of the solvent was placed first in the trough and supplemented during irrigation. The solvent for irrigation was butanol-pyridine-water (5 :3 :2) solution. In this case, aniline hydrogen phthalate

Fig. 1

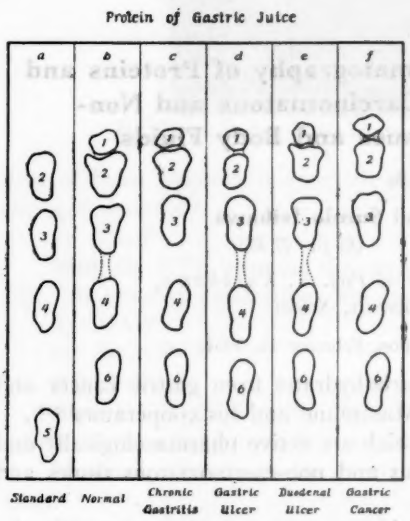

Fig. 2

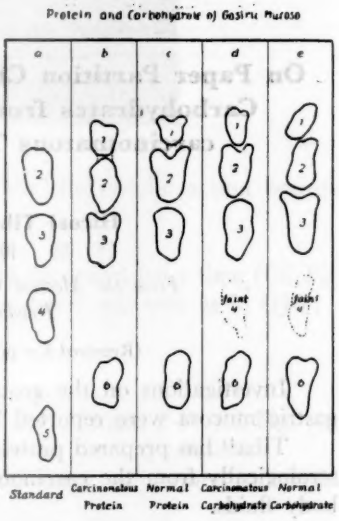

Fig. 1. Butanol-pyridine-water (5 :3 :2) chromatograms of the hydrolysate of proteins in gastric juice. Indicator: aniline hydrogen phthalate of Partridge. Duration of test, 24 hrs, temp., 27°±1°C.

a: standard, b: normal gastric juice protein, c: chronic gastritis juice protein, d: gastric ulcer juice protein, e: duodenal ulcer juice protein, f: gastric cancer juice protein. 1: chondrosamine, 2: glucosamine, 3: galactose, 4: mannose, 5: rhamnose, 6: l-fucose.

Fig. 2. Butanol-pyridine-water (5 :3 :2) chromatograms of the hydrolysate of proteins and carbohydrates in gastric cancer and gastric mucosa. Indicator, duration of test and temperature same as in Fig. 1.

a: standard, b: gastric cancer protein, c: normal mucosa protein, d: gastric cancer carbohydrate, e: normal mucosa carbohydrate.

Ciphers express the same as in Fig. 1.

in butanol (reagent of Partridge[6]) was used as an additional developer.

Materials

1) Preparations of the proteins from gastric juice of 5 groups, namely normal, chronic gastritis, gastric ulcer, duodenal ulcer and gastric cancer.

2) Preparations of the proteins from gastric cancer and normal gastric mucosa. These were prepared formerly by Tiba[4].

3) Preparations of the carbohydrates from gastric cancer and gastric mucosa.

(a) Carbohydrate of gastric cancer, (C-ch). Tumor tissue was dissected from gastric cancers and cut into small pieces to preserve in about

Fig. 3

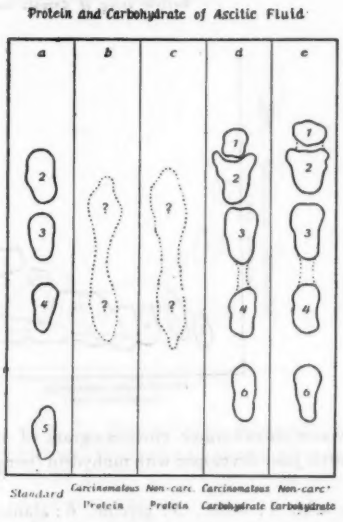

Fig. 4

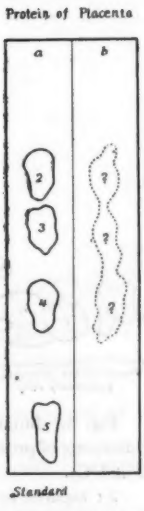

Fig. 3. Butanol-pyridine-water (5 :3 :2) chromatograms of the hydrolysate of proteins and carbohydrates in carcinomatous and non-carcinomatous ascitic fluids. Indicator, duration of test and temperature same as in Fig. 1.

a : standard, b : carcinomatous protein, c : non-carcinomatous protein, d : carcinomatous carbohydrate, e : non-carcinomatous carbohydrate.

Ciphers express the same as in Fig. 1.

Fig. 4. Butanol-pyridine-water (5 :3 :2) chromatograms of the hydrolysate of placental protein. Indicator, duration of test and temperature same as in Fig. 1.

a : standard, b : protein of placenta.

Ciphers express the same as in Fig. 1.

7 volumes of 95% alcohol for the purpose of dehydration. 150 g. of the tissue thus dehydrated was masticated, dried in a vacuum desiccator (H_2SO_4) and pulverized in a mortar. Fibers removed, the remaining powder amounted to 34 g. The preparation proceeded as follows:

It was stirred with 20 volumes of water for 3 days, using toluene as a preservative, and centrifuged. The residue was treated likewise three times, until no more substance came out. The centrifuged supernatant liquids collected were distilled to about 200 cc. To that extract was added the saturated $Ba(OH)_2$ to cause maximum precipitation (the pH was shifted to 9.0) and centrifuged. The supernatant liquid was acidified by 30% acetic acid to pH 4.2, and precipitates centrifuged. After these processes, barium was taken out with just sufficient amount of sulfuric acid

Fig. 5

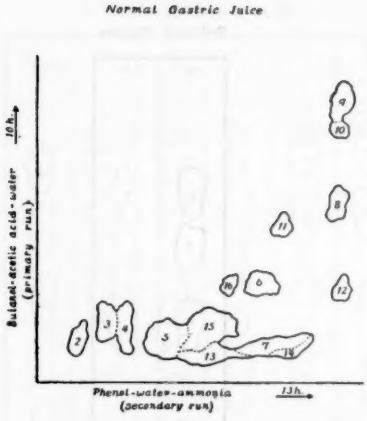

Fig. 6

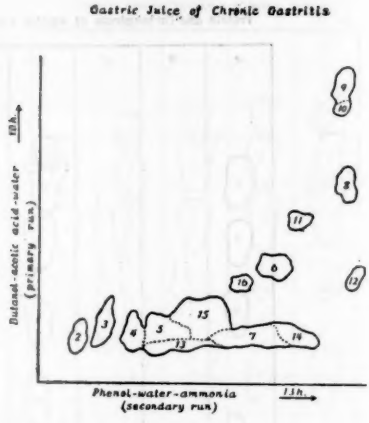

Fig. 5. Butanol-acetic acid-water/phenol-water chromatogram of the hydrolysate of protein in nomral gastric juice developed with ninhydrin (temp., 20°±1°C).

2: aspartic acid, 3: glutamic acid, 4: serine, 5: glycine, 6: alanine, 7: histidine, 8: valine or methionine, 9: leucine or isoleucine, 10: phenyl-alanine, 11: tyrosine, 12: proline, 13: lysine, 14: arginine, 15: hexosamine, 16: threonine.

Fig. 6. Butanol-acetic acid-water/phenol-water chromatogram of the hydrolysate of protein in gastric juice due to chronic gastritis. 2–16 signify the corresponding numbers in Fig. 5.

(1 *N*). The centrifuged supernatant liquid was made neutral with NaOH (0.05 *N*). To this liquid $(NH_4)_2SO_4$ was added at 1/2 saturation of the salt (20°C), and slight turbidity appeared.

The powder of $(NH_4)_2SO_4$ was added to it at full saturation (20°C) that precipitation was most evident. It stood at 20°C for 30 minutes. The flocculent precipitate was centrifuged off. The supernatant liquid was dialyzed through cellophane against running water for 3 days and condensed to a syrup, precipitated with alcohol and dried. The dried material was dissolved in 20 cc. of water and the powder of $(NH_4)_2SO_4$ at full saturation was added. It stood for about 30 minutes. The transparent solution freed from some precipitates that appeared was dialyzed for 3 days. It was then eliminated from some precipitate which had appeared, and 10% lead acetate was added until the maximum precipitation took place. It was centrifuged and filtrated through a creased paper. H_2S gas passed sufficiently through the filtrate to take off lead completely. The precipitation was filtrated through a creased paper. The filtrate was dis-

Fig. 7

Fig. 8

Fig. 7. Butanol-acetic acid-water/phenol-water chromatogram of the hydrolysate of protein in gastric juice due to gastric ulcer.
2–16 signify the corresponding numbers in Fig. 5. and 17 expresses under-glutamic acid (?).

Fig. 8. Butanol-acetic acid-water/phenol-water chromaotgram of the hydrolysate of protein in gastric juice due to doudenal ulcer.
2–17 signify the corresponding numbers in Figs. 5 and 7.

tilled to a syrupy consistency, precipitated with alcohol and dried.

The product was taken up in 50 cc. of water and electrodialyzed (180 Volts) for 24 hours. The trace of precipitation that had appeared was centrifuged off. The supernatant liquid was distilled, precipitated with alcohol, and dried. The white powder was given.

b) Carbohydrate of gastric mucosa (M-ch). Carbohydrate of gastric mucosa was treated in a similar manner as in the above (C-ch).

Chemical properties

Qualitative assays gave the following results:

	C-Ch	M-Ch
Biuret	−	−
Molish	##	##
Ninhydrin	±	±
Phosphorus	−	−
Neuberg-Saneyoshi	−	−

±: faintly positive, ##: strongly positive, −: negative.

Quantitative analysis. C-Ch.: Nitrogen (micro Kjeldahl) 4.3%, hexosamine (Blix method[7]) 36.6%., ash 0.8%.

M-Ch: Nitrogen 4.6%, hexosamine 34.8%, ash 1.3%.

Fig. 9

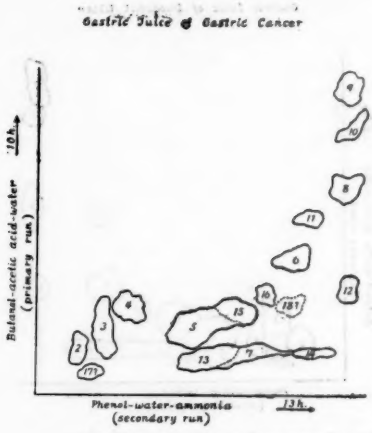

Fig. 10

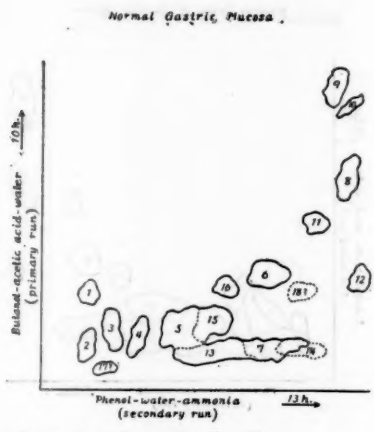

Fig. 9. Butanol-acetic acid-water/phenol-water chromatogram of the hydrolysate of protein in gastric juice due to gastric cancer.

2–17 signify the corresponding numbers in Figs. 5 and 7, and 18 expresses an unknown spot.

Fig. 10. Butanol-acetic acid-water/phenol-water chromatogram of the hydrolysate of protein in normal gastric mucosa.

1 : cysteic acid, 2–18 : signify the corresponding numbers in Figs. 5, 7 and 9.

4) Preparations of the proteins and carbohydrates from the carcinomatous and non-carcinomatous ascitic fluids.

These materials were prepared by the procedure of Tiba *et al.*[8] at the Medico-chemical Institute of Prof. H. Masamune.

5) Preparation of the protein from placenta.

This was reported in the previous paper[4].

In all the cases hydrolysis of the materials and subsequent preparation of the solution for chromatography was carried out as follows :

30 mg. in the cases of carbohydrates, 50–100 mg. in the cases of proteins from the specimens mentioned above were hydrolyzed with 3–5 cc. of 1 *N*. H_2SO_4 in an ampoule by heating at 100°C for about 5 hours. The hydrolysates nearly neutralized till pH 5.8 with baryta and the supernatant liquid was condensed to about 0.5 cc. From this condensate 0.02 and 0.03 cc. portion was sampled.

Results

As Figs. 1 and 3 (d, e) show, proteins from gastric juice of 5 groups and carbohydrates from ascitic fluids contained chondrosamine, gluco-

Fig. 11

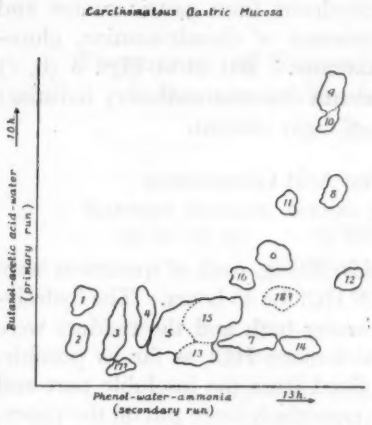

Fig. 12

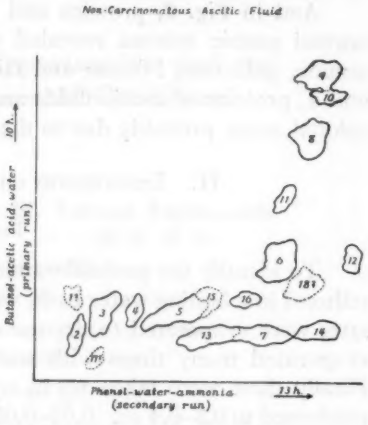

Fig. 11. Butanol-acetic acid-water/phenol-water chromatogram of the hydrolysate of protein in gastric cancer. Ciphers express the same as in Fig. 10.

Fig. 12. Butanol-acetic acid-water/phenol-water chromatogram of the hyd o'ysate of protein in non-carcinomatous ascitic fluid.

Ciphers express the same as in Fig. 10.

Fig. 13

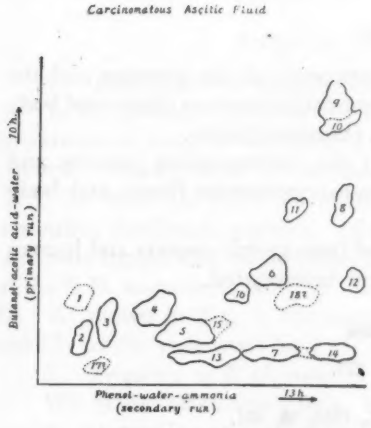

Fig. 14

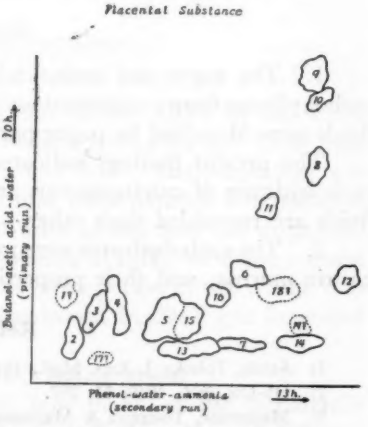

Fig. 13. Butanol-acetic acid-water/phenol-water chromatogram of the hydrolysate of protein in carcinomatous ascitic fluid.

Ciphers express the same as in Fig. 10.

Fig. 14. Butanol-acetic acid-water/phenol-water chromatogram of the hydrolysate of placental protein.

1–18 signify the same as in Fig. 10 and 19 expresses an unknown spot.

samine, galactose, mannose and l-fucose.

And in Fig. 2, proteins and carbohydrates from gastric cancer and normal gastric mucosa revealed the presence of chondrosamine, glucosamine, galactose, l-fucose and faint mannose. But as in Figs. 3 (b, c) and 4, proteins of ascitic fluids and placenta demonstrated very indistinct colored areas, probably due to the lack of sugar content.

II. Experiments on Amino Acid Components

Technique

To identify the probable amino acids, 30 mg. each of specimens were refluxed in a boiling water bath, with 6 *N* HCl for 48 hours. The hydrolysates were evaporated to dryness on a water bath and the residues were evaporated many times with water to remove HCl as far as possible. Finally these were taken up in water, freed from the insoluble part and condensed to 0.3–0.4 cc. 0.05–0.08 cc. respectively were put on the paper, and primarily run (ascending) with butanol-acetic acid-water (4 :1 :5), secondarily run with phenol-water (4 :1) in an atmosphere saturated with ammonia.

Results

Figs. 5–14 illustrate the aquired chromatograms diagramatically.

Summary

1. The sugar and amino acid components of the proteins and the carbohydrates from carcinomatous and non-carcinomatous tissues and body fluids were identified by paper partition chromatography.

The present findings indicated that the corresponding proteins and carbohydrates of carcinomatous and non-carcinomatous tissues and body fluids are resembled each other.

2. The carbohydrates were prepared from gastric cancers and human gastric mucosa, and their properties were investigated.

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Frequency of Essential Hypertension and the Influence of Environmental Condition thereon

By

Katsuya Itahara, Setsuo Fukuchi, Tokuya Fujibayashi
(板原 克哉) (福地 攝男) (藤林 徳也)
and **Motoo Yamaguchi**
(山口 素夫)

From Prof. Nakazawa's Internal Clinic of Medical Department, Tohoku University, Sendai

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The authors have made a series of investigations to find out what was the frequency of the incidence of asymptomatic essential hypertension (referred to as E. H. hereunder, for short) in our native-born population and the relation of the said frequency with the environmental conditions. Prior to the above investigations, we observed the numerical change of the E.H. patients in the inpatients of our clinic in the later years of World War II, when the food condition in Japan fell to the lowest level.

Frequency of E.H. Incidence

1) *Investigation on the in-patients*

In the period of 13 years from Jan. 1937 to Dec. 1949, we had 7,272 in-patients in total in our clinic, 4,318 of whom were men and 2,954 were women. Among them 151 or about 7% had E.H. The percentage was 8.1% for men and 5.5% for women, so that male patients outnumbered somewhat the female patients. Next, in order to find how the worst food condition imaginable would affect the frequency of E.H., we made a count of the E.H. in-patients in every year in the latter period of the World War II. As shown in Fig. 1, the number was lowest in 1947, but increased steadily as the year went by.

2) *Frequency of E.H. found in apparently healthy persons*

We made an investigation to find how many of the apparently healthy persons in active life were suffering from E. H. About 6,000 men and women above 20 years of age working in various offices, factories and department stores in Sendai were examined for this purpose. We took as our working rule that a systolic blood pressure above 150 mm. indicated hypertension. As our investigation covered a large number of people,

Fig. 1. Annual variations of the number of E. H. patients. Ordinates: Number of E. H. patients admitted to Nakazawa's Clinic. Abscissae: The year, A. D.

adjustment according to the age of the individual subjects was impracticable, so we had to set up such a fixed standard, which may be looked upon as somewhat lenient toward youth and rather strict for older generations. We also examined the fragility of the capillaries of all the examinees who had a blood pressure of over 150 mm., by means of an improved Wright-Lilienfeld's method[1]. The results were given in our tables under the abbreviation W-L and those showing more than 11 petechias were regarded as fragile in their capillaries.

The frequency of E.H. incidence varied with the kind of occupation of the examinees, ranging from 3% at the lowest and to 9.3% at the highest, averaging in total at 5.3%. Fragility of capillaries also showed such a tendency to vary, but the total average stood at 1.5%. The present population of Japan is estimated as something around 80 million, of which some 43,200,000 are above 20 years in age, according to the latest census. If the above percentage is applicable to the whole population of Japan, we would have 2,290,000 with too high blood pressure and 650,000 with fragile capillaries. The figures are quite imposing.

The above investigation covered men and women between 20 and 60 years of age. When only those of 40 and more years of age are considered, the average frequency of asymptomatic hypertension rises to 11.9% and of capillary fragility to 4.4%.

Next, an investigation with 3,883 students between 19 and 29 years of age as objectives revealed that 3.5% of them had asymptomatic hypertension and 0.6% had fragile blood vessels. According to the investigation of U.S.A. college students by Diehl and Sutherland[2], 5.6% of them had hypertension. In this respect, the U.S.A. students take a lead over the Japanese students.

3) *Investigation at agrarian villages in Akita Pref. noted for their high percentage of apoplexy cases, and at fishing villages in Iwate Pref. with very low percentage thereof*

The agrarian villages in the basin of the River Omono in Akita Prefecture are known for their high percentage of apoplexy victims—indeed the highest, not only in Japan, but throughout the world. On the contrary, some secluded fishing villages on the Pacific shore in Iwate Prefecture have had the lowest percentage of cerebral hemorroage in Japan. In both cases, the apoplexy is mainly occasioned by high blood pressure. From June to July in 1950, we conducted a collective investigation covering about 9,000 natives of more than 20 years of age, both male and female, living and working in these villages. Concerning the hypertensive with a blood pressure exceeding 150 mm., we made a special study on the subjects of the exact percentage of the frequency, the fragility index of their vessels, their response to cold pressor test, their urine reactions, their heredity, food conditions and proclivities for alcoholic drinks and tobacco, the rice production status and labor condition in the districts, the winter life and such environmental living conditions.

In the villges situated along the River Omono, 30% of the inhabitants were found to have hypertension, while some fishing villages in Iwate Prefecture had less than 10% of hypertension patients among their natives. However, in the same Akita Prefecture, some maritime villages on the Oga Peninsula showed the low frequency of 5% of hypertensive subjects, while in some inland districts, in Iwate Prefecture, where more rice is produced, the frequency rises to 20%. Such statistics seem to indicate that hypertension is more prevalent in regions rich in rice production than otherwise.

The Effect of Environmental Conditions on the Occurrence of E.H.

1) *Climate and season*

According to the researches of Oda[3], and Mitutani and Isa[4], hypertension is rare among the natives and the Japanese population in tropical Taiwan, while Chamberlain[5] reports that the Americans living in the Philippines have lower blood pressure than those living in the States. Ohashi[6] says that the blood pressure is inversely related to the climate.

Japan is very long in the direction of the meridian, with warm south and cold north. Therefore, we chose four cities for our next stages of investigation, Kagoshima City as the warmest, Wakayama as the moderately warm, Sendai as the moderately cold and Hachinohe as the coldest samples in climate.* Hokkaido was excluded from our plan as including too many immigrants from other prefectures in its present population, though of course it is the coldest region in all Japan. In the early summer we carried out an investigation on the frequency of E.H. in these four localities, taking persons of over 20 years of age in active life in their respective home cities. As Table I shows with surpassing clarity, the frequency of E.H. is higher in the colder regions.

TABLE I

Comparison of the Occurrence of Hypertension at Warm and Cold Localities

City	Number of persons examined	Occurrence (%) of hypertension
Kagoshima	667	1.4
Wakayama	922	2.7
Sendai	5101	5.3
Hachinohe	1287	9.4

In our next investigation, the same investigator had to measure the precise blood pressure of 246 workers, including male and female, in a same department store in Sendai, twice in the same year, once in the hot June and once in the cold December. The results were subjected to close statistical analysis (Table II), and it was found that though the diastolic

TABLE II

Season and Elevation of Blood Pressure

Systolic pressure

N	$\bar{\Delta}$	u^2	Difference
246	3.983	144.084	Significant

Diastolic pressure

N	$\bar{\Delta}$	u^2	Difference
246	1.0203	162.248	Insignificant

$\bar{\Delta}$ Difference in average.

* We express our sincere thanks to Dr. H. Sato of Kagoshima University, Dr. K. Kusui of Wakayama University and Dr. K. Ishibashi of Hachinohe Red Cross Hospital, for their assistance in our investigations.

blood pressure registered no significant difference in summer and winter, the systolic blood pressure showed a quite significant high of 1% in December. At Kagoshima, Dr. H. Sato made the same test and obtained a similar result. From such observations, we can explain, at least partially, why cerebral hemorrhage occur more frequently in winter months, for cold temperature may be looked upon as one of the factors that cause hypertension. Probably, this is due to the fact that the autonomous nervous system, the endocrinal secretion and the tension of the blood vessels themselves are affected by cold temperature, but on the other hand, we hear that the Esquimaux living in the Arctics and the Scandinavians living in the coldest regions in Europe do not suffer more from hypertension, so we must admit that coldness is only one of the many environmental factors fostering hypertension.

2) *Effect of diets rich in rice and salt*

The region lying in the basin of the River Omono in Akita Prefecture is very rich in rice production and the inhabitants are accustomed to consume much rice daily together with very salty courses as side-dishes. The salty dishes are the naturally demanded appetizer for the rice gourmands. This district, as mentioned above, is known for its high frequency in hypertension and cerebral hemorrhage. In the coastal district in Iwate Prefecture cited above as showing a very low frequency in high blood pressure, rice production is meagre and over-salty dishes are not much in vogue. These facts were what arrested our attention as we made our investigations in these regions. We conclude that consumption of too much rice and table-salt is also one of the factors favoring hypertension.

3) *Effect of smoking and drinking*

There is no experimental foundation proving the harm of smoking and drinking alcoholic liquors, but it is generally believed that these habits are conducive to hypertension. It is still uncertain whether smoking and drinking directly prompt hypertension or act indirectly by complicating heart, kidney or brain troubles which are apt to accompany hypertension. In the coastal districts of Iwate Prefecture, where the average life-span is long, there are few old agers who do not relish saké (a medium-strength liquor brewed from rice) and tobacco.

1) Drinking saké. The results of our examinations on the frequency of hypertension among the saké bibbers and abstainers in the agrarian and fishing villages in Akita, Iwate and Miyagi Prefectures are shown in Tables III and IV. In the tables, the marks (+) show light, the marks (++) medium and the marks (+++) heavy drinkers. The subjects were divided into those above and below 40 years of age. Table III shows the result of a similar investigation with women, and no difference of frequency of any significance is apparent between drinkers and non-drinkers. Table IV a)

TABLE III

Occurrence of Hypertension in Drinkers and Non-drinkers (♀)

Locality	Non-drinker or drinker		Age 20-30 y.			Age 40 y.-		
			Persons examined	Hypertensive persons	% of occurrence	Persons examined	Hypertensive persons	% of occurrence
a) Akita-Pref.	Non-drinker		106	41	38.67	235	158	67.23
	Drinker	+	12	4	33.33	119	85	71.42
		++	0	0	0	8	6	75.00
		+++	0	0	0	4	3	75.00
		Total	12	4	33.33	131	94	71.67
b) Iwate-Pref.	Non-drinker		140	11	7.86	232	110	47.41
	Drinker	+	4	1	25.00	31	18	59.03
		++	0	0	0	2	1	50.00
		+++	0	0	0	0	0	0
		Total	4	1	25.00	33	19	57.57
c) Miyagi-Pref.	Non-drinker		302	36	11.92	264	126	42.69
	Drinker	+	10	3	30.00	39	19	48.73
		++	0	0	0	4	2	50.00
		+++	0	0	0	1	0	0
		Total	10	3	30.00	44	21	47.72
a)+b)+c)	Non-drinker		548	88	16.05	731	394	53.89
	Drinker	+	26	8	30.76	189	122	64.55
		++	0	0	0	14	9	64.28
		+++	0	0	0	5	3	60.00
		Total	26	8	30.76	208	134	64.42

+-++-+++ Measure of "Saké" drinking.

shows the result of investigation with men in Akita, b) in Iwate and c) in Miyagi Prefectures. , In apparence, the frequency seems higher among saké drinkers, but upon closer statistical analysis, it is proved that there is no significant difference here also. Upon a wider-scoped study of the statistics by taking all the data given in the three tables in one, it was found that the frequency of hypertension in men (Table IV) of over 40 years of age is higher by the quite significant value of 5% among the drinkers than among the abstainers. Therefore, we may be justified in regarding the habit of drinking liquor as one of the factors prompting high blood pressure, but we must not be too quick in concluding that liquor is such a big factor in hypertensionism as is commonly believed.

In the next place, is it true that the drinking habit fosters

TABLE IV

Occurrence of Hypertension in Drinkers and Non-drinkers (♂)

Locality	Non-drinker or drinker		Age 20-39 y. Persons examined	Hypertensive persons	% of occurrence	Age 40 y.- Persons examined	Hypertensive persons	% of occurrence
	Non-drinker		18	5	27.78	37	18	48.64
a) Akita-Pref.	Drinker	+	28	6	21.02	97	67	69.07
		++	17	7	41.17	62	46	74.19
		+++	13	4	30.76	45	34	75.55
		Total	58	17	29.31	204	147	72.05
	Non-drinker		23	3	13.04	49	13	26.53
b) Iwate-Pref.	Drinker	+	37	2	5.40	79	25	31.64
		++	8	0	0	30	14	46.67
		+++	6	0	0	11	7	63.64
		Total	51	2	3.92	120	46	38.33
	Non-drinker		38	6	15.79	55	18	32.73
c) Miyagi-Pref.	Drinker	+	23	2	8.65	39	12	30.77
		++	20	4	20.00	24	11	45.83
		+++	15	4	26.67	17	9	52.94
		Total	58	10	17.24	80	32	40,00
	Non-drinker		79	14	17.72	141	49	34.75
a)+b)+c)	Drinker	+	88	10	11.36	215	104	48.37
		++	45	11	24.44	116	71	61.20
		+++	34	8	23.52	73	50	68.49
		Total	167	29	17.36	404	225	55.69

+-++-+++ Measure of "Saké" drinking.

fragility of blood vessels and susceptibility to cold pressor? Tables V and VI seem to indicate that the answer is negative for both the effects. At least, there is no remarkable influence noticeable either on the capillary fragility or on the cold pressor test.

ii) Smoking. We separated our subjects into the groups of smokers and non-smokers and statistically compared the respective frequency of hypertension among the two groups, as shown in Table VII. In this table, (—) shows non-smokers, and (+), (++) and (+++) the light, medium and heavy smokers respectively. Upon statistical examination, we found no significant difference in the frequency index among the groups.

We made also a comparative study of the groups anent their showing in the tests on the fargility of blood vessels and cold pressor response

TABLE V

"Saké" and Fragility of Blood Vessels

Locality	Sex	Non-drinker or drinker		Persons examined	Persons whose W-L more than 11	
					Number	%
Akita-Pref.	♂	Non-drinker		78	29	37.2
		Drinker	+	125	38	30.4
			++	99	35	35.4
			+++	55	17	30.9
	♀	Non-drinker		383	185	43.3
		Drinker	+	118	72	61.0
			++	6	1	16.0
			+++	2	2	100.0
Iwate-Pref.	♂	Non-drinker		44	14	31.8
		Drinker	+	51	20	39.2
			++	32	11	34.4
			+++	38	10	26.3
	♀	Non-drinker		213	115	54.0
		Drinker	+	18	13	72.2
			++	0	0	0
			+++	0	0	0

(Tables VIII & IX), but again we could prove no significant difference.

Judging from the above, we think the habits of smoking and drinking are not such a powerful factor in begetting or fostering hypertension, as is vaguely but commonly believed, though they may be harmful to the heart, kidney and brain troubles secondary to hypertension in so many cases.

4) *Body weight and heavy excercise*

The "Rikishi" or the Japanese wrestlers are excessively heavy-weighted, are generally addicted to heavy eating and drinking, must undergo rigorous training all through the year and otherwise live in very special environments. In the summer of 1950 we examined 82 rikishis of age between 16 and 35 and of weights between 142 and 360 lbs. We found no parallelism between their body weights and blood pressure. Then we examined their blood pressure before and immediately after their violent training excercise and noted that the systolic blood pressure rose in one half of the examined rikishis and dropped in the other half, the latter slightly out-numbering the former, so that it was impossible to find a de-

TABLE VI

"Saké" and Cold Pressor Effect

Locality	Sex	Non-drinker or drinker		Persons examined	Increase of blood pressure more than 20 mm	
					Number	%
Akita-Pref.	♂	Non-drinker		63	24	31.8
		Drinker	+	133	68	51.1
			++	103	38	36.9
			+++	56	23	41.1
	♀	Non-drinker		363	126	34.7
		Drinker	+	133	37	27.8
			++	7	2	28.6
			+++	2	1	50.0
Iwate-Pref.	♂	Non-drinker		55	5	9.1
		Drinker	+	37	6	16.2
			++	30	5	16.7
			+++	31	5	16.1
	♀	Non-drinker		184	6	3.3
		Drinker	+	29	2	6.9
			++	1	0	0
			+++	0	0	0

TABLE VII

Occurrence of Hypertension in Smokers and Non-smokers

Sex	Non-smoker or smoker		Age 20-39 y.			Age 40 y.-		
			Persons examined	Hypertensive persons	%	Persons examined	Hypertensive persons	%
♂	Non-smoker		94	17	18.08	286	122	51.69
	Smoker	+	108	19	17.59	232	123	53.01
		+++	44	7	15.90	77	29	37.66
♀	Non-smoker		612	99	11.17	931	498	53.49
	Smoker	+	14	4	28.57	72	40	55.55
		+++	1	0	0	8	5	62.50

finite tendency in any direction.

In short, in these examinations we failed to prove any direct relation between body weight and blood pressure or any definite tendency in the

TABLE VIII

Smoking and Fragility of Blood Vessels (♂)

Locality	Measure of smoking	Persons examined	W-L more than 11	
			Number	%
Akita-Pref.	−	171	65	38.0
	+	134	34	25.4
	++	52	21	40.4
	+++	7	3	42.8
Iwate-Pref.	−	66	23	34.9
	+	63	25	39.7
	++	36	12	33.3
	+++	22	8	36.4

TABLE IX

Smoking and Cold Pressor Effect (♂)

Locality	Measure of smoking	Persons examined	Increase of blood pressure more than 20 mm	
			Number	%
Akita-Pref.	−	162	72	44.4
	+	140	68	48.6
	++	47	21	44.7
	+++	6	4	66.7
Iwate-Pref.	−	59	6	10.1
	+	58	8	13.8
	++	33	7	21.2
	+++	3	0	0

influence of heavy excercise upon blood pressure, which seemed only temporary and ambiguous.

5) *Effect of syphilis*

The Wassermann's reaction of the 511 hypertensive in-patients who have been in our wards in the past thirteen years was examined and we had 8.9% of positive reactors by blood test and 4.9% by test of cerebrospinal fluid. So, it seems syphilis has a very slight relation, if any, with high blood pressure. Hiramoto[7] says syphilis does not cause hypertension, but if E.H. accompanies syphilis, the hypertension tends to aggravate. Our research also proved that 12.6% of the hypertensive who died in the 13 years under examination showed positive reaction in Wassermann's test.

6) *Strain of civilized life*

No hypertension is found among the negroes living in their jungles in

Africa, but among the descendants of old negro immigrants in civilized U.S.A., there are many hypertensives. There are also many among the Jews, so that some call hypertension a civilization disease. But if hypertension is a civilization disease caused by overexertion, luxury and unnatural living exacted by civilization, it is strange to see prevalence of hypertension in the rural villages in the basin of the River Omono, where civilization is very low. At any rate, a strained life is not enough as a factor by itself to heighten blood pressure.

7) *Comparative study of environmental conditions in some agrarian villages in Akita Prefecture and fishing village in Iwate Prefecture*

The largest percentage of hypertension incidence in Japan is found in some agrarian villages in Akita Pref. and the lowest in some fishing villages in Iwate Pref. The former district also showed higher marks in our capillary fragility and cold pressor tests (Tables X & XI). In consideration of these facts, we will proceed to study the environmental conditions in these two districts in comparison.

i) The agrarian districts in Akita Pref., as most localities on the Japan Sea, are under the influence of Siberia in their climate, overcast almost all through the year and cold and snowy in winter. The coast of Iwate Pref., on the other hand, is washed by the warm Kuroshio current and

TABLE X

Comparison of Fragility of Blood Vessels in Akita and Iwate Prefecture

Locality	Age (y.)	Persons examined	W-L more than 11	
			Number	%
Akita-Pref.	20-29	34	8	23.5
	30-39	29	13	44.8
	40-49	39	19	48.7
	50-59	76	57	75.0
	60-69	42	35	83.3
	70-	24	20	83.3
	Total	244	152	62.3
Iwate-Pref.	20-29	72	0	0
	30-39	35	1	2.9
	40-49	51	6	11.8
	50-59	38	5	13.2
	60-69	24	6	25.0
	70-	8	6	75.0
	Total	228	24	10.0

TABLE XI
Comparison of Cold Pressor Effect in Akita and Iwate Prefecture

Locality	Sex	Persons examined	Increase of blood pressure more than 20 mm	
			Number	%
Akita-Pref.	♂	355	163	45.5
	♀	505	166	32.9
	Total	860	331	38.5
Iwate-Pref.	♂	153	20	13.1
	♀	214	8	3.7
	Total	367	28	7.6

the climate is comparatively mild even in winter, though it is on the same latitude with Akita.

ii) In the agrarian villages in Akita Pref., noted for their large rice production, the inhabitants are addicted to a diet almost entirely consisting of an overdose of boiled rice, seasoned with a dish or two of salty courses, while in the fishing villages on the rugged coast of Iwate Pref., they have not much rice to eat, and the side-dishes usually contain more fresh fish-meat and sea weed, which are not oversalted.

iii) The agrarian villages in Akita Pref. are also known for the illicit home-brewed "Doburoku" or unrefined saké, where women and even children are often rather stout drinkers, while in the fishing villages in Iwate Pref., rice production is meagre and "doburoku" brewing is not prevalent. In the latter district they have to buy licensed and refined saké if they have the inclination and the means thereto. Consequently, liquor consumption is much lower in the latter than in the former.

iv) In the Akita villages they have much snow in winter and the residents are confined indoors for nearly four long months. Much fuel wood is burned under bad ventilation, so that the air is contaminated with much CO and CO_2, a condition very harmful for the cardiovascular system. Moreover, in these winter months, the only pastime consists in visiting each other in their ill-ventilated houses, where they foregather at the smoky firesides, and pass nights in drinking much "doburoku" and eating much over-salted pickles. Not so with the fishermen on the Pacific coast of Iwate. Here, the winter months are the season for salmon fishing, so they spend their time outdoors in healthy labor, and the weather is usually favorable enough for such an out-door life.

v) In the Akita villages, in-marriages between boys and girls of the same village is the custom, with consequent fortification of the hereditary predisposition to hypertension in the descendants.

In summarizing such observations, we have to admit that the environmental conditions in the rural life of Akita villages are more favourable to hypertension than those in Iwate. Among them, the oversalted side dishes, we take it, are the worst factor in causing hypertension in combination with several other factors.

Conclusion

I. The frequency of essential hypertension (E.H.) incidence :—

1. The number of E.H. patients in our wards showed a very remarkable drop in the last years of World War II, when the food conditions in Japan fell to the lowest level.

2. In 1950, when the food condition in Japan recovered the level of the pre-war period, there were found, among some 6,000 men and women of over 20 years of age working in various positions and outwardly and apparently healthy, about 5.3% of cases with more than 150 mm. in blood pressure reading, and 1.5% with marked fragility of capillary blood vessels.

3. The region comprising the agrarian villages in the basin of the River Omono in Akita Pref. is reported as having the highest mortality rate by cerebral hemorrhage not only in the mortality statistics of Japan but also in those of the world. Among the native-born active population of these villages, the frequency of hypertension was found to amount to 30%.

II. Influence of environmental conditions upon the occurrence of E.H. :—

1. The incidence to hypertension tends to rise in cold climate or season.

2. Much rice and table-salt in the diet may promote occurrence of high blood pressure.

3. Smoking and drinking liquor cannot be looked upon as a major factor in fostering hypertension, as has been vaguely but widely believed hitherto.

4. Syphilis is not directly related with high blood pressure, but when a hypertensive contracts syphilis, the hypertension tends to be aggravated.

5. Civilized life under strain is not necessarily a powerful factor leading to hypertension.

6. The authors have subjected the environmental conditions prevalent in some agrarian villages in Akita Prefecture where the frequency of hypertension is registered as the highest, and in some fishing villages in Iwate Prefecture where the frequency is the lowest in Japan, to a comparative study from various points of view.

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On the Heredity of Essential Hypertension

By

Shoichiro Uchigasaki

(内ヶ崎 尚一郎)

From Prof. Nakazawa's Internal Clinic of Medical Department, Tohoku University, Sendai

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According to Frant and Groen's report[1], heart attack is mentioned as occupying the first place in the cause of deaths among hypertensive patients in U.S.A., followed by uremia at a wide gap, the last in the order being cerebral hemorrhage. As a step in initiating a similar study in Japan, I sent letters of inquiry to the families of 511 hypertensive in-patients who left our clinic as adequately recovered for extra-hospital life in these 15 years, to which I received 199 answers in total. By this method of inquiry and answer, I ascertained that a majority of 60% of deaths of the hypertensive was caused by cerebral hemorrhage in Japan, secondly by heart attack in a far lesser extent and in a small minority by uremia. That is to say, the majority of the hypertensive in Japan die of cerebral hemorrhage, or in other words, the victims of cerebral hemorrhage are in their majority hypertensive.

The hereditary transmission of the tendency to essential hypertension (referred to as E.H. hereunder for short), that is, that many of the hypertensive have or had some near relative suffering or deceased of E.H. or cerebral hemorrhage, has been a fact recognized by many students on the subject. However, it is not yet established whether E.H. may be classed as one of the genuine hereditary defects as defined by the geneticians of the orthodox school, as, for example, the color-blindness, and in practice, we find cases of E.H. who are apparently free of any such hereditary weakness.

In consideration of such a situation, I undertook a thorough study on the relation of E.H. and heredity in the agrarian district in the basin of the River Omono in Akita Pref., known for the highest frequency of E.H. in Japan, and in some fishing villages in Iwate Pref., where the E.H. frequency is at the minimum.

Investigation on heredity in agrarian and fishing villages. In the district along the River Omono in Akita Pref. about 80% of the villagers had apoplexy in their ancestors and in the other rice producing areas of the

same Pref., 80–70% of the inhabitants showed hereditary tendency to E.H., while in the coastal villages of Iwate Pref., the frequency was something less than 40%. Next, the number of hypertensive who denied any heredity asserting absence of cerebral hemorrhage cases in their families was investigated. In the agrarian villages in Akita Pref., the number was only 10%–20% of the total hypertensive, showing the scarcity of non-hereditary hypertensives, while in the fishing villages in Iwate Pref., the percentage was somewhat higher, standing at 20–60%. How is this rather marked difference to be explained? I would attribute the difference to the dissimilar environmental conditions prevailing in the two regions. In the basin of the River Omono, the climate and the food condition are liable to cause hypertension, while in the coastal villages in Iwate Pref., the climate is much milder, fish and seaweed are more abundant, and less rice is consumed. The environmental conditions are evidently much healthier in the latter district. In the latter district, therefore, hypertension, if contracted or inherited, would not aggravate or end fatally in apoplexy as frequently as in the Omono River Basin, and many of the hypertensive live to attain high age, as I could ascertain in my practical investigation. This is also the reason why there are so few cases of apoplexy in this district, and as a result, there was so large a percentage of hypertensives who were unaware of any hereditary tendency to E.H. in their families.

Heredity in younger generations. I chose an agricultural school in the basin of River Omono in Akita Pref. where 30% of the inhabitants have hypertension and another in Miyagi Pref. in a district similar to the former in topography, environmental conditions and status in rice production, and examined all the students in these two schools (boys of 15 to 20 years of age) for the percentages of those who had apoplexy in their families, of those asymtomatically hypertensive, of those with fragile blood vessels and with sensitive response to cold pressor test. The results indicated that 73% in the Akita school and 24% in the Miyagi school showed heredity in their families, 29.2% in the former and 3.3% in the latter hypertension, 2.4% in the former and 0.4% in the latter fragile blood vessels and 31.7% in the former against only 8.9% in the latter sensitivity against cold pressor test, respectively. The fact that young men who had not yet lived long under the unhealthy environmental conditions show hypertensive tendency indicates that these predispositions to E.H. are largely hereditary.

Statistical analysis. The results of comparative study on the percentage of hypertensive persons who had heredity and those who had no heredity, based on the statistics compiled upon the collective investigations made in Akita, Iwate and other localities, are shown in Table I. The percentage is significantly higher among those who showed evidence of heredity.

Examination of heredity pattern of E.H. Assuming that E.H. is intensively hereditary, there are some studies on the pattern of the heredity (Nikami and Nagahama[2], Miyao[3]), but the question whether it is dominant or recessive in character is not yet solved. In the following, I will mention my study of a group of hypertensive families (Fig. 1).

TABLE I

Occurrence of E. H. in Native-born People in Akita and Iwate Prefecture

Age	Heredity	Number examinee	Hypertensive persons	Percentage
20-39	+	552	111	20
	−	681	56	8
40-	+	1455	803	55
	−	862	324	37

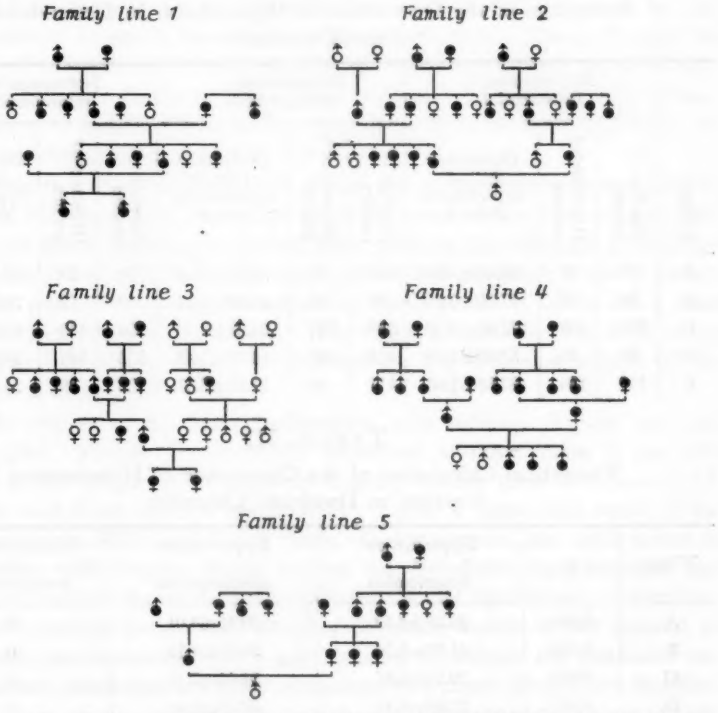

Fig. 1. Some examples of family lines in Akita Prefecture. ♀ : Apoplexy or hypertension. ♀ : Neither apoplexy nor hypertension.

Upon investigating these family lines, I must say that the heredity as appeared in their genealogy is apparently dominant. Assuming E.H. to be a hereditary disease, we computed the frequency of E.H. by actual calculation of cases in 688 families which had been subjected to a precise investigation in this respect as far as possible. Next we calculated the theoretical frequency as based on the Mendelistic statistical method, both as dominant and recessive characters, and compared the result with the values obtained by the above actual calculation and tried to obtain a clue to the question whether E.H. is dominant or recessive.

Table II shows the results of the actual investigation, and Table III the theoretically computed values on the assumption that E.H. heredity is dominant. Comparing these tables, we find many points of coincidence, but the theoretical deduction that, if both the parents are normotensive, their children would not show hypertension, is inconsistent with actual

TABLE II

Percentage of the Occurrence of Hypertension in Descendants (actual number)

Village	Hypertension × hypertension			Hypertension × normotension			Normotension × normotension		
	Number of couples	Hypertensive persons	Occurrence (%) of hypertension from F_1	Number of couples	Hypertensive persons	Occurrence (%) of hypertension from F_1	Number of couples	Hypertensive persons	Occurrence (%) of hypertension from F_1
A	67	42	62.68±5.90	149	78	52.34±4.08	74	29	39.18±5.67
B	58	25	47.16±6.85	144	66	45.38±4.14	56	15	26.23±5.87
C	206	150	72.81±3.10	533	330	61.91±2.10	304	143	47.03±2.86
D	89	46	51.68±5.29	418	187	44.73±2.43	558	181	32.43±1.97
E	153	134	87.58±2.66	141	45	31.91±3.97	138	29	21.01±3.46

TABLE III

Theoretical Calculation of the Occurrence of Hypertension assumed as Dominant Character

Village	q	Hypertension × hypertension	Hypertension × normotension	Normotension × normotension
A	0.682	83.56±4.52	59.45±4.01	0
B	0.762	81.30±5.34	56.75±4.12	0
C	0.837	79.24±2.82	54.44±2.15	0
D	0.897	77.64±4.41	52.71±2.44	0
E	0.871	78.33±3.31	53.45±4.19	0

TABLE IV

Theoretical Calculation of the Occurrence of Hypertension assumed as Recessive Character

Village	q	Hypertension × hypertension	Hypertension × normotension	Normontension × normotension
A	0·730	100	42.20±4.03	17.80±4.43
B	0.647	100	39.28±4.06	15.43±4.82
C	0.545	100	35.27±2.06	12·47±1.89
D	0.440	100	30.55±4.87	9.34±1.22
E	0.490	100	32.88±3.94	10.81±2.36

observations. Table IV shows the theoretical values of the case when E.H. is of recessive character. Again, we find many points in coincidence with the factual calculations listed in Table II, but the theoretical deduction that, if both the parents are hypertensive, all their children must be also hypertensive, is again inconsitent with actual facts. Thus, though the pattern of heredity of E.H. is seen to show kinship with both the dominant and the recessive characters, it is not thoroughly consistent with either. This seems to indicate that E.H. is greatly influenced in its frequency by environmental conditions beside heredity.

From the above investigation, I am led to the following conclusion. Heredity may be said to constitute an important basis for hypertension, but before this defect becomes apparent, there must be the influence of environmental conditions exerted over many years, which gradually tends to undermine the patient's capacity for adjusting blood pressure, till at last a state of patent hypertension is reached. Therefore, I conclude that E.H. is not a genuine hereditary disease as defined by the orthodox school of the students of heredity.

The relation of hereditary predisposition with capillary fragility and cold pressor effect. Firstly, I statistically examined whether there is any difference in the frequency of capillary fragility among those who have E.H. heredity and those who do not have it. Table V shows the result of the examination in Akita and Iwate Prefs. In appearance, the table seems to show more with fragile vessels among the hereditary hypertensive, but statistical analysis shows that the difference is not significant. Therefore, though I cannot decide that fragility of blood vessels is not related to hereditary predisposition, yet I am inclined to believe the postnatal environmental conditions are more efficacious as a cause of capillary fragility.

Next, a similar comparison was made concerning the effect of cold pressure. Table VI shows the result of the investigation in some farm-

TABLE V

Heredity and Fragility of Blood Vessels (W-L)

Locality	Sex	Heredity	Number examined	Cases in which W-L more than 11	
				Number	%
Akita Prefect.	♂	−	35	20	36.4
		+	235	71	30.2
		++	67	34	50.7
	♀	−	88	39	44.3
		+	330	170	51.5
		++	91	51	56.1
Iwate Prefect.	♂	−	74	22	29.8
		+	81	30	37.0
		++	10	5	50.0
	♀	−	102	50	49.0
		+	119	67	56.3
		++	10	7	70.0

TABLE VI

Heredity and Cold Pressor Effect (Akita Prefect.)

Sex	Heredity	Number examined	Increase of systolic blood pressure more than 20 mm.	
			Number	%
♂	−	61	21	34.4
	+	222	106	47.7
	++	72	38	52.8
♀	−	79	17	21.5
	+	327	117	35.8
	++	99	32	32.3

villages in Akita Pref. Statistical analysis of this result shows that the difference is quite significant in that the E.H. hereditary show more cases of sensitivity against cold pressor test. Table VII shows the result of the investigation conducted in some fishing villages in Iwate Pref. No significant difference is observable here. Only the E.H. hereditary in Akita farm-villages are apparently more sensitive against cold pressor test in average. This difference, I presume, is again attributable to the different environmental conditions in the two districts. Hereditary predisposition in itself is not enough to affect cold pressor effect; harmful environmental

TABLE VII

Heredity and Cold Pressor Effect (Iwate Prefect.)

Sex	Heredity	Number examined	Increase of systoric blood pressure more than 20 mm	
			Number	%
♂	−	65	9	13.8
	+	73	10	13.6
	++	15	2	13.3
♀	−	93	1	1.1
	+	11	4	36.3
	++	10	3	30.0

conditions must supervene. Appearance of intense cold pressor effect has been looked upon as indicative of prehypertensive state by Brown[4], who laid great importance on it.

As the final step in my series of investigations, I chose about 10,000 persons for examination in Akita, Iwate and Miyagi Prefs. and divided them into several groups—those who had apoplexy or hypertension in both their parents, in one of their parents, in their uncles and aunts, in one of their grandparents, and those who had no records of hypertension in their family lines. Then, I found about 50% of hypertensives among those who had apoplexy or hypertension in both their parents, about 40% among those who had these diseases in one of their parents, about 35% among those who had hypertensives or apoplectics among their uncles and aunts, 27% among those who had one such among their grandparents. 22% of the group of the apparent non-hereditary were hypertensive. In U.S.A., says Ayman[5], the percentage of hypertension among the children with parents both hypertensive is 35%, among those with one hypertensive parent only, 28.3%, and among the non-hereditary, 3.1%. In comparison, I must say the frequency of hypertension is higher in Japan than in U.S.A., especially, among the non-hereditary.

CONCLUSION

1. In Japan, about 60% of the hypertensive die of apoplexy.

2. Among the inhabitants of the agrarian villages situated in the basin of the River Omono in Akita Pref., 80% of them have records of cerebral hemorrhage cases among their ancestors, while of the inhabitants in some fishing villages in Iwate Pref., only 40% had such genealogical records. In the former district, non-hereditaries counted only 10–20% among the hypertensive, but in the latter district, 20–60% of the hyper-

tensive claimed non-heredity. The reason of this discrepancy seems to lie in the fact that the environmental conditions are incomparably more conducive to longevity and prevention of cerebral hemorrhage in the latter, so that the deaths, even of hypertensives, have been attributed to simple senility, and the hereditary tendency for hypertension remained concealed.

3. Even among the younger generations, who have not yet suffered much from unhealthy environmental conditions, various investigations have proved the prevalence of hereditary predisposition.

4. E. H. shows a considerable heredity, which seems to be dominant in character, but the influence of the environmental conditions over a long period apparently plays such an important role, that I must hesitate in concluding E.H. to be a hereditary disease in its orthodox sense, when the results of strict genetico-statistical study are taken into consideration.

5. The results of the investigation with about 10,000 Japanese as objective, show that the percentages of hypertensives appearing in descendants are as listed in the following:

When hypertension or apoplexy is recorded		
In both parents	Approximately	50%
In one of the parents	,,	40%
In one or some of the uncles or aunts	,,	35%
In one of the grandparents	,,	27%
In none of the ancestors	,,	22%

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On Hemagglutinin in Antipneumococcal Type XIV Horse Serum*

By

Hajime Masamune and **Zensaku Yosizawa**
(正宗 一) (吉澤 善作)

From the Medico-chemical Institute (Director: Prof. H. Masamune)

and

Tosiyuki Miki
(三木 敏行)

From the Forensic Medical Institute (Director: Prof. T. Murakami), Tohoku University, Sendai

(Received for publication, March 8, 1954)

In 1938 Finland & Curren[1] discovered the occurrence of agglutination of human red cells of all four blood groups by type XIV antipneumococcal horse serum, and in the following year, Goebel *et al.*[2] and Weil & Sherman[3] worked on this subject. Goebel & others' results suggested that the type XIV specific polysaccharide (SXIV) contanis, in addition to the type specific structure, a structure in common with the Group A mucopolysaccharide which precipitated more than half of the total antibody in the serum reacting with SXIV. In the experiments of Weil & Sherman, the hemagglutinating factor of the serum was completely absorbed by the type-specific carbohydrate of type XIV and on the other hand, the serum agglutinated not only human erythrocytes and stromata but also rabbit and cat erythrocytes. Furthermore, human organs (heart, kidney and liver) absorbed the hemagglutinating quality from the serum.

Later on Kabat made with his co-workers[4)-8)] repeated studies of that hemagglutination in connection with the structural relationship between the blood group substances within the ABO classification and the type XIV pneumococcus polysaccharide. These authors found that all of A, B and O human and hog blood group mucopolysaccharides cross-reacted with the antiserum and that the cross-reactivity was increased by controlled acid hydrolysis of the group substances. Since SXIV consists of acetylglucosamine and galactose[2] and the group mucopolysaccharides contain L-fucose

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in addition to those sugar components, Kabat put forward the opinion that a series of N-acetylglucosamine-galactose residues, possessing at various points partial structures similar to those in the chain of SXIV, forms the main chain of the group substances attached with L-fucose molecules projecting outward at various points. According to him, the L-fucose residues prevent close approach of the antibody molecules to the reacting groupings on the main chain so that removal of them by the partial hydrolysis provides cross-reacting sites. By the hydrolysis the group substances lost the specific activity and therefore he said that " the labile amino acid ", which is split off thereby," may possibly be responsible for conferring blood group activity on the galactose-glucosamine polysaccharide, since .... the fucose (or at least some of it) is not necessary for blood group activity."

More recently, Annison & Morgan[9] considered more probable that the agglutination of human erythrocytes by the antiserum is due to components of the red cell surface other than A, B and H(O) receptors such as the product of the Le^a gene. The reason was that the group substances of ABO type obtained from human ovarian cysts failed to react with the antibody but the Le^a substance from the same source did react. They thought the latter to be a material containing a carbohydrate possibly with basic glucosamine-galactose structure, which according to Kabat is responsible for the cross-reactivity mentioned, being based on the indirect evidence afforded by a periodate oxidation experiment on erythrocyte surface. The Group A and H substances from ovarian cysts, which did not precipitate with the antiserum, gave the reaction after mild acid hydrolysis causing scission of L-fucose[14][15] from the carbohydrate moiety. The authors assumed the A and H group substances above to be similar to those in red cells, despite that the group substances in erythrocytes are compound lipids[11]-[13] containing hexosamine and hexose but no L-fucose.

We undertook to scrutinize those studies with a horse serum against antipneumococcus type XIV (Kabat's H-618) which Prof. Kabat kindly supplied.[16] At first, precipitin test of the serum was tried with a Group A substance obtained after alkaline cleavage at 20°C of pig stomach mucus and its products after heating with acetic acid, whose preparation procedures and analyses have been already reported (Chs. I, II and III in an article of Masamune & Yosizawa[17]). As is shown in Table I, no positive precipitin reaction occurred with any of Chs. I, II and III which were Group A active up to dilutions of $1:6.4\times10^5$, $1:4\times10^4$ and $1:4\times10^4$ respectively and Group O active (against normal anti-O goat serum diluted to titre 4) up to dilutions of $1:6.4\times10^4$, $1:8\times10^3$ and $1:10^3$ respectively. The preparations contained glucosamine, galactose aud L-fucose, the methylpentose content decreasing from left to right in the order arranged that Ch. III contained only 0.8% fucose. Even Ch. I was a scission product

of a natural group substance and the alkali-labile fragments split off during preparation (Cf. Masamune, Yosizawa and Miki[18]) may have a structure reacting with the hemagglutinating antibody, nevertheless, the finding above furnishes at least an evidence that configurations not related to the type XIV pneumococcus polysaccharide can develop group action.

TABLE I

Precipitin Test of the Type XIV Antipneumococcal Horse Serum with Chs. I, II and III

The ring test was applied. The results of the test were judged after 5 hours from the overlaying. −: negative reaction. Ch. I: a group mucopolysaccharide separated from pig stomach mucus by scission with 1 *N* NaOH at 20°C. Chs. II and III: products from Ch. I obtained after heating at 100°C with 1 *N* CH_3COOH for 1 and 3 hours respectively.

Ch.	Dilution of the antigens (1 :)					
	10^2	10^3	10^4	10^5	10^6	10^7
I	−	−	−	−	−	−
II	−	−	−	−	−	−
III	−	−	−	−	−	−

In the next, absorption of the horse serum was processed with type XIV pneumococcus, erythrocytes and saliva of various groups separately, followed by determination of agglutinin titre for erythrocytes. The results will be described item by item.

TABLE II

Occurrence of Hemagglutination by the Type XIV Antipneumococcal Horse Serum treated with Type XIV Pneumococcus

Various amounts of type XIV pneumococcus were suspended in 1 cc. of physiological saline and 1 cc. portions of the serum diluted 2.5-fold were absorbed with the suspensions. The serum portions absorbed were progressively doubled with physiological saline and 1 drop was transferred from each of them onto a glass slide, followed by addition of 1 drop of a 1% erythrocyte suspension. The mixtures were occasionally shaken at room temperature and the results were judged after 30 minutes. The figures in the table express agglutinin titres.

Erythrycytes	Unabsorbed serum	Amount of the bacteria taken for absorption (mg.)		
		2	5	8
Ae (1)	160	40	10	no aggl. at 5-fold dilution
BE (1)	320	40	10	"
O (1)	320	40	10	"

1) The agglutinin titres of the antiserum toward various groups of erythrocytes lowered to equal values stepwise by absorption with increasing amounts of the pneumococci, whose lyophile[16] was also given from Prof. Kabat (Table II). Although the titre of the serum unabsorbed was somewhat lower toward A erythrocytes than toward the others, the consequence allows the possibility that the agglutination of the different groups of erythrocytes is caused by one and the same agglutinin.

2) AS, BS, OS and Os salivas gave no positive precipitin reaction with the serum (Table III), but all of them absorbed the hemagglutinin

TABLE III

Precipitin Test of the Type XIV Antipneumococcal Horse Serum as such with Salivas of Individuals belonging to Different Groups

The procedure of the test was the same as that in Table I. — : negative reaction.

Saliva	Dilution of salivas (1 :)									
	1	3	10	3×10	10^2	3×10^2	10^3	3×10^3	10^4	∞
OS (1)	—	—	—	—	—	—	—	—	—	—
Os (1)	—	—	—	—	—	—	—	—	—	
AS (1)	—	—	—	—	—	—	—	—	—	
As (1)	—	—	—	—	—	—	—	—	—	
BS (1)	—	—	—	—	—	—	—	—	—	
Bs (1)	—	—	—	—	—	—	—	—	—	

(Tested with O erythrocytes) (Table IVA) notwithstanding that the secretor salivas only absorbed O erythrocyte agglutinin from an eel serum (Table IVB). Thus the agglutinin in the horse serum was found removable by a factor or factors other than the group substances which are owned by A, B and O salivas, that have a structure which, judging from the present findings together with those of Weil and Sherman, is common to human, rabbit and cat.

3) With the conclusion above do not conflict following results which resembled those of the predecessors. As seen in Table V, erythrocytes belonging to a particular group decreased the hemagglutinin potency for various erythrocytes to similar extents, regardless of their blood group and strength of E potency.

In short, it might be claimed that the hemagglutinin in question is produced by the XIV pheumococcus polysaccharide and combines with carbohydrate-containing substances, which have a configuration common to human, rabbit and cat but have nothing to do with group activity.

TABLE IV

Occurrence of Agglutination of Erythrocytes by the Type XIV Antipneumococcal Horse Serum and an Eel Serum both after Treatment with Salivas of Various Groups

To 0.2 cc. portions of each saliva, doubled progressively with physiological saline, was added 0.2 cc. of one of the antiserums diluted to agglutinin titre 8. The mixtures were stood at 37°C and at room temperature for 1 hour each and in a refrigerator (0°C) overnight in turn. Then 1 drop was transferred from each of the mixtures onto a glass slide to examine the hemagglutinating potency of the serum as in the experiment of Table II. O erythrocytes were used in the test here. − : no agglutination, + : slight aggl., ++ : not-marked aggl.

Saliva used for absorption	Dilution of salivas for absorption (1 :)					
	1	2	4	8	16	32
A. Hemagglutination by the horse serum absorbed						
OS (2)	−	−	+	+	+	
Os (1)	−	+	+	+	+	
AS (2)	−	+	+	+	+	
BS (2)	−	+	+	+	+	
B. Hemagglutination by the eel serum absorbed						
OS (2)	−	−	−	−	−	+
Os (1)	+	+	+	+	+	++
AS (2)	−	−	−	−	+	+
BS (2)	−	−	−	−	+	+

SUMMARY

1. A specimen of Group A substance (contaminated with some Group O substance), obtained after alkaline scission at 20°C of pig stomach mucus, as well as the products from it after conditioned acid hydrolysis did not precipitate with an antipneumococcus type XIV horse serum.

2. The horse serum lost the agglutinating potency toward various groups of erythrocytes to equal extents when absorbed with type XIV pneumococcus.

3. The hemagglutinin in the horse serum was absorbed by secretor and non-secretor salivas in similar grades.

4. The horse serum treated with erythrocytes of one blood group lost the potency of agglutinating various groups of erythrocytes in similar degrees.

We were led from the findings above and those of former investigators to the conclusion that the hemagglutinin in the type XIV antipneumococcal

TABLE V

Hemagglutination by the Type XIV Antipneumococcal Horse Serum treated with Erythrocytes of Various Groups

The examination was processed in a similar manner as described in Table II. The figures express agglutinin titres.

A. The serum was absorbed with AB erythrocytes

Here the serum was diluted 5-fold prior to absorption.

Erythrocytes* for determining aggl. titre	Before absorption	Group and volume of erythrocytes used for absorption			
		ABe (1)		ABE (2)	
		1/5 volume	additional 1/5 vol.	1/5 vol.	add. 1/5 vol.
ABE (1)	320			20	no aggl. at 5-fold dilution
ABe (1)	320	20	no aggl. at 5-fold dilution	20	"
ABE (2)	160			10	"
ABE (3)	320			20	"
ABe (2)	160	10	"	10	"
O (1)	320	20	faint aggl. at 5-fold dilution	20	faint aggl. at 5-fold dilution

* The AB erythrocyte samples in the arranged order were agglutinated by an eel serum at dilutions of 1 : 200, 1 : 10, 1 : 200, 1 : 200 and 1 : <10 respectively.

B. The serum was absorbed with O, A or B erythrocytes

The serum was absorbed without prior dilution.

Erythrocytes for measuring aggl. titre	Before absorption	Group and volume of erythrocytes used for absorption					
		O*		A*		B*	
		1/2 volume	additional 1/10 vol.	1/2 vol.	add. 1/10 vol.	1/2 vol.	add. 1/10 vol.
Ae (1)	160	4	no aggl.	4	no aggl.	4	no aggl.
BE (1)	320	4	"	4	"	4	"
O (1)	320	4	"	8	"	8	"

* Collected from several persons.

horse serum reacts with carbohydrate-containing factors other than the A, B and O group substances with a surface structure common to species human, rabbit and cat.

Through the Grant Committee for Scientific Researches was given a grant from the Education Department in aid to us. H. Masamune.

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Studies of High-molecular Placental Components in Connection with Pregnancy Toxemia. Third Paper On Placental Desoxypentosenucleic Acid (DNA)*

By

Yosizi Mugikura
(麥倉義司)

From the Medico-chemical Institute (Director: Prof. H. Masamune) and the Department of Obstetrics and Gynecology (Director: Prof. T. Shinoda), Tohoku University, Sendai

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For the etiological study of pregnancy toxemia along our line, Kanno *et al.*[1] and Kanno[2] extracted the components from placentas, once dehydrated with alcohol, by boiling with water. However, their preliminary treatment of placentas and manner of extraction both do not appear appropriate to obtain the substances for the aim of biological examination, because the natural highly-associated state of them is possibly lost thereby. Hence, we have made changes that the fresh tissue is extracted immediately and further the products, whose solubility decreases on isolation even by freeze-drying, are applied to the biological experiments at the stage where they are free from impurities but still in solution.

The present account deals with the DNA as the first example according to the new plan. Judging from the results of animal experiments, this component is possible to take some part in causing pregnancy toxemia.

Experimental

Preparation of the DNA

3 fresh placentas, washed with water and freed from blood vessels and membranes, were cut into small pieces, and after squeeze, ground in a masticator. The brei obtained weighed 464 g. The material was shaken with 1.5 volumes (700 cc.) of 0.14 *M* NaCl containing 0.01 *M* sodium citrate, under cooling in ice for 24 hours. The extraction was carried out twice more with 650 and 550 cc. of the citrate-containing saline. The solutions were used for separating proteins, mucopolysaccharides and

* The 6th report of Masamune and coworkers' "Nucleic Acids."

pentosenucleic acid. More repetitions of the operation with 0.14 *M* NaCl of the tissue are not advisable, because the yield of DNA is lowered. Thereafter, the tissue residue was treated by replacing 10% NaCl (also containing 0.01 *M* sodium citrate) for the 0.14 *M* NaCl in a similar manner to above. 3 successive solutions here given were subjected separately to the following operations exemplified with the first solution. This reddish, turbid and extremely viscous solution amounting to 600 cc. was percolated through a gauze and to the filtrate was added ice-cooled water in small portions to 0.9–1.0% NaCl, under agitation with wooden chopsticks. The fibrous precipitate wound round the sticks (Dische ++, biuret ++, Molisch ++, P ++) was taken out and after again dissolving in 10% NaCl (30 cc.) by means of a homogenizer, placed in a refrigerator (0°C). The yield from the second solution was less and that from the third was so slight that nearly all the DNA of the tissue appeared to have been exhausted. The solutions of the DNP portions were combined (60 cc. in total) and precipitated by dilution with water as above. Dissolution and precipitation was repeated 3 times more, when the deposit (Purified DNP) was non-colored and cotton-waste-like and gave only weak Molisch reaction. Dische ++, biuret ++.

The product was kneaded well, without anterior drying, with some ice-cooled 10% NaCl, and then after filling up to 150 cc. with this solvent, agitated in a homogenizer. To the solution was added an equal volume of 91% alcohol. And the non-colored fibrous precipitate was separated by means of a glass rod and purified by 4 reprecipitations in which case the substance came out as shorter and shorter threads. The substance given—Crude DNA-Na—was Molisch-negative, slightly biuret-positive and strongly Dische-positive. It recovered to an aggregation state giving a precipitate in long threads, when its solution in 10% NaCl (containing sodium citrate) was kept at 0°C for several days.

The product here was dissolved again in 10% NaCl by means of a hemogenizer, and after standing in a refrigerator (0°C) overnight, treated with a chloroform-amylalcohol mixture (4:1 by volume) according to Sevag again and again, until no more gel formed. The solution was then precipitated with 2 volumes of abs. alcohol. The occurring fibrous deposit was taken out with a glass rod and dissolved in water by means of a homogenizer as above. The non-colored, transparent, viscous solution was dialyzed against running tap water for 1 week and followingly against changes of ice-cooled distilled water for 1 day, and precipitated with 3 volumes of abs. alcohol containing some sodium acetate. The deposit was washed with alcohol and ether in turn and dried in vacuo over $CaCl_2$. 201 mg. of a white cotton-like substance were given—Purified DNA.

Physical and Chemical Properties of the Purified Preparation of the DNA

Qualitative test. Biuret, Molisch, Goldschmiedt and Neuberg-Saneyoshi negative; Bial almost negative, Dische and phosphorus- and purine-test markedly positive.

Precipitability. Its solution in water was precipitated by baryta, lead acetate and cupric chloride.

Quantitative analysis. N (micro Kjeldahl) 13.7%; P (Haneda, Sinokawa & Yasuoka's modification of Plimmer method) 7.7%; (N:P= 1.7); ash (Pregl) 22.0%; bases (Masamune & Sakamoto[3])[3a]: adenine 9.9%, guanine 7.2%, cytosine 5.5%, thymine 8.9%, (Molecular ratios calculated to a total of 4 are 1.22:0.79:0.82:1.17). Uracil and 5-methylcytosine were not detected.

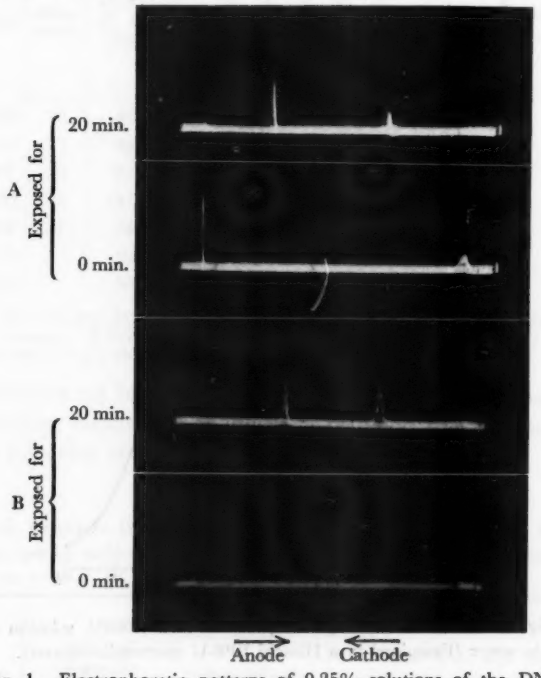

Fig. 1. Electrophoretic patterns of 0.25% solutions of the DNA in phosphate buffer pH 7.7, I 0.2 (A) and acetate buffer pH 4.5, I 0.2 (B); temperature 20°C; current 8.3 and 8.0 mA respectively; mobility in the phosphate buffer: $\frac{1.20\times0.0087\times0.3}{1200\times0.0083}=31.4\ \mathrm{cm^2\cdot volt^{-1}\cdot sec^{-1}\cdot 10^5}$, mobility in the acetate buffer: $\frac{1.21\times0.0067\times0.3}{1200\times0.008}=25.3\ \mathrm{cm^2\cdot volt^{-1}\cdot sec^{-1}\cdot 10^5}$.

Electrophoresis. A single boundary was demonstrated after electrophoresis of it both at pH 7.7 and at pH 4.5, as is shown in Fig. 1.

Viscosity. It was measured at 25°C in an Ostwald viscosimeter, with results:

Conc. in water %	Time of outflow	η_r
0	62.6 sec.	
0.175	19 min. 6.8 sec.	18.3
0.0875	4 min. 57.5 sec.	4.8
0.05	2 min. 38.2 sec.	2.5
0.025	1 min. 42.8 sec.	1.6

Ultraviolet absorption. A 0.004% solution in water gave the spectrum in Fig. 2 (max. absorption: λ 260 mμ, ε(P) (atomic extinction coefficient with respect to phosphorus)[4] 7204; min. absorption: λ 232.5 mμ, ε(P) 3202).

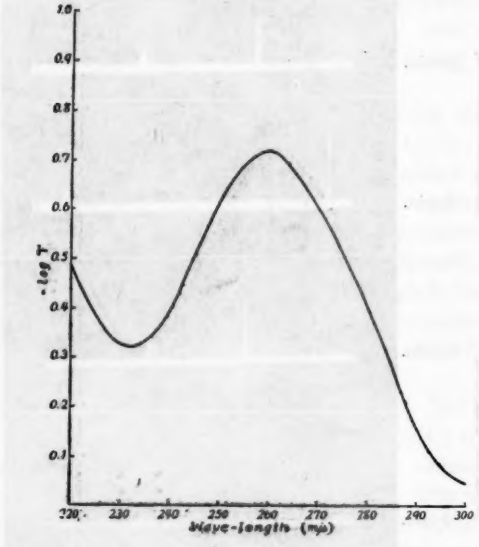

Fig. 2. Ultraviolet absorption spectrum of a 0.004% solution of the DNA in water (Examined in a Hitachi EPB-U spectrophotometer).

Biological Assays

These were carried out by Drs. Funakubo and Saito by the techniques described in the first communication of this series of investigation[1]. The results are only summarized below, because the details will be published by those workers.[5][6]

1) Pathological changes in liver and kidney of pregnant rabbits after intravenous injection of the DNA. See Table I.

TABLE I

Pathological Lesions in Liver and Kidney of Pregnant Rabbits after Injection of the DNA into Auricular Vein

5 mg. per kg. body weight of the substance (as a 0.38% solution in physiol. saline) were injected. $-\sim\!\!+\!\!+$ with respect to histological changes signify the same grades of the changes as the corresponding notations in the paper of Kanno *et al.*[1]

Rabbit No.	Rabbit Wt.(g.)	Days of pregnancy	Time of killing animal aft. inj. (hrs.)	Liver: Necrosis	Liver: Necrobiosis	Liver: Cloudy swelling	Liver: Degeneration	Liver: Stasis of blood str.	Liver: Thrombosis	Liver: Change of Glisson capsule§	Kidney: Nephrosis	Kidney: Glomerulonephrosis	Premature delivery//	Aggrav.¶ of general status aft. inj.
62	3080	20	46	−	−	+	++	+	−	++	−	−	+	+
63	3130	27	46	−	−	±	+	±	−	+	−	−	+	+
64	2800	25	47	−	+‡	+	+++	−	−	+	−	−	+	++
65	2530	19	47	−	+‡	−	++	−	−	−	−	−	−	+
66*	2280	21	34*	−	−	+	+	−	−	+	±	−	−	++
Contr. 1†	2150		48	−	−	+	−	−	−	++	+	−		+
Contr. 2†	2540		50	−	−	+	−	−	−	−	−	−		±

* Died after 34 hrs. from the injection. † A non-pregnant female rabbit was injected. ‡ Anemic. § Cellular infiltration and swelling of the sheath. // + signifies the occurrence. ¶ ±: faint, +: slight, ++: marked.

2) Influence on blood pressure of pregnant rabbits. As is shown in Table II, neither elevation nor lowering of blood pressure occurred after intravenous injection of the substance.

TABLE II

Blood Pressure in Carotic Artery after Injection of the DNA

5 mg. per kg. body weight of the DNA (a 0.38% solution) were injected into auricular vein.

Rabbit No.	Rabbit Wt.(g.)	Days of pregnancy	Blood pressure (mm. Hg)					
			Before injection	Time after injection (min.)				
				0.5	1	3	5	7
67	2530	19	86	90	83	84	82	83
68	2280	21	110	112	109	112	110	
Control*	2150		120	117	117	112	114	

* A non-pregnant female rabbit was injected.

3) No change of urine volume as compared with the control in non-pregnant female rabbits nor albuminuria occurred, when 5 mg. per kg. body weight of the substance (as a 0.38% solution) were injected into auricular vein of urethane-narcotized pregnant rabbits, into whose stomach 100 cc. of luke-warm water were administered simultaneously.

4) Precipitin reaction. The substance gave the positive reaction at dilutions of 1:250, 1:500 and 1:500~1,000 respectively with 1 of the sera of 8 non-pregnant women, 2 of those of 8 normal-pregnant and 2 of those of 7 pregnancy-toxemic, with some tendency of more strong occurrence of the reaction in pregnancy toxemia than in normal pregnancy.

In short, the findings in liver and kidney of the pregnant rabbits in 1) and the result of the precipitin test suggested that the placental DNA plays a rôle in causing pregnancy toxemia, although it is much less effective in comparison with certain proteins from the same source as will be described by Yosizaki[7].

Through the Grant Committee for Scientific Researches the Education Department gave us a grant for defraying the expenses of this work, which is gratefully acknowledged. H. Masamune and T. Shinoda.

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Note at proof-reading: Funakubo's article (5)) has been published in that Journal (1955, **7**, 89).

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Biochemical Studies on Carbohydrates

CLXXIV. Analysis of Mutarotation of N-Glycosides

First Paper

On Aniline-N-Lactoside

By

Shigeru Tsuiki

(立木 繁)

From the Medico-chemical Institute, Tohoku University, Sendai; Director: Prof. H. Masamune

(Received for publication, March 8, 1954)

Regarding rotation change of free sugars, the opinion has prevailed that it is due to anomerization. On the contrary, however, that of N-glycosides has been often ascribed to isomerization of the lactol ring, for instance, Howard *et al.*[1] said that, of the two modifications of 2-nitro-4,5-dimethyl-aniline-L-arabinoside which Kuhn & Ströbele[2] had prepared and assumed as furanosides, one must be in fact a pyranoside. Berger & Lee[3] could obtain two isomers of aniline-D-riboside by condensation of aniline and D-ribose under different conditions and considered one of them to be a furanoside and the other to be a pyranoside. More recently, Weygand *et al.*[4] expressed a similar claim regarding two isomers of *p*-nitroaniline-D-xyloside.

As early as 1918, Haworth & Leitch[5] observed mutarotation of the anilide of 2,3,4,6-tetramethyl-galactopyranose. In this case, isomerization of the type pyranoside⇄furanoside can not occur, and therefore that of the $\alpha\beta$-type appears most probable. However, the analysis of the change of rotation of N-glycosides of free sugars is not an easy problem in general, because the N-glycosidic link is extremely unstable against the chemical agents which serve the purpose of establishing the ring structure, and hence, with the aim in view, the writer picked out at first aniline-N-lactoside which is blocked at C_4 of the glucose residue.

In 1888, Sorokin[6] prepared aniline-lactoside by heating its components with 96% ethanol. The product had $[\alpha]_D$ in water of $-14.19°$. The writer could not prepare in all his attempts the lactoside identical with Sorokin's but a product (**I**) with F.P. of 199–201° and $[\alpha]_D^{20}$ in dry pyridine of $-82.1°$ by the procedure of Weygand[7], and a second (**II**) with F.P. of 159° and $[\alpha]_D^{20}$ in dry pyridine of $-13.5°$ by rapid precipitation with dry

ether of a methanolic solution of the components heated for a long time. On standing for many days in air, or when recrystallized, whether from dry or aqueous methanol, without use of ether, **II** changed into **I**. Thus **I** proved to be the more stable isomer than **II**, but **I** also changed into **II** when carried down with dry ether after long heating in dry methanol. Hence, **I** and **II** are interconvertible. Next, Ellis & Honeyman[8] pointed out that the more dextrorotatory isomer of aniline-riboside (Berger and Lee's aniline-N-α-ribofuranoside[3]) is obtained, only when water is strictly avoided throughout the whole course of preparation. To synthesize **II** above, however, it was requisite not only to condense the components in perfectly water-free medium but also to add a large volume of dry ether at one time for crystallizing out of the product.

Since aniline-N-lactoside can not take furanoid structure of the glucose residue, its rotatory change takes place by simple anomerization, provided that hydrolysis and Schiff base formation are excluded. Schiff base can be detected by polarography, and its formation was disproved in any of the solvents employed in this work (0.01 *N* NaOH, buffers of pH 5.6, 7.0 and 9.6). (Regarding the polarography, details will be described in Second Paper.[8a]) In 0.01 *N* NaOH, hydrolysis of the lactoside was found also not to occur by determination of free aniline (See Experimental). In this solvent, **I** and **II** rotated to the end value of $[\alpha]_D^{20}$ of $-42°$ from the original $-65.7°$ and $-7.3°$ respectively in about 3 hours (Tables IA and IB and Fig. 1). According to Hudson[9], the mutarotation velocity of the $\alpha\beta$ interconversion of free sugar pyranoses follows the equation of the first order reversible reaction:

$$k_1+k_2=\frac{1}{t}\log\frac{r_o-r_\infty}{r_t-r_\infty},$$

where k_1 and k_2 are the respective reaction constants of $\alpha\rightarrow\beta$ and inverse rearrangements, and r_o, r_t and r_∞ the respective rotations at the beginning, at time t and at equilibrium. If the mutarotation of the writer's materials in 0.01 *N* NaOH owes its entire to establishment of $\alpha\beta$-isomerization, k_1+k_2 values calculated from the rotation changes of the isomers must equal each other, and $\log(r_t-r_\infty)$ values plotted against t fall on two parallel straight lines. In fact, the equation above accurately held true here, as is revealed by k_1+k_2 values (0.0085) in Tables IA and IB and the log (r_t-r_∞) (r signifies $[\alpha]_D$) curves in Fig. 2, in other words, **I** is concluded to be the β isomer and **II** the α isomer. k_1 was calculated at 0.0046 and k_2 at 0.0039.

Effect of H and OH ions upon the rotation change of aniline-N-lactoside. Mutarotation of free sugars is catalyzed by OH ion more intensively than by H ion, but the relation between the ions is said to be the con-

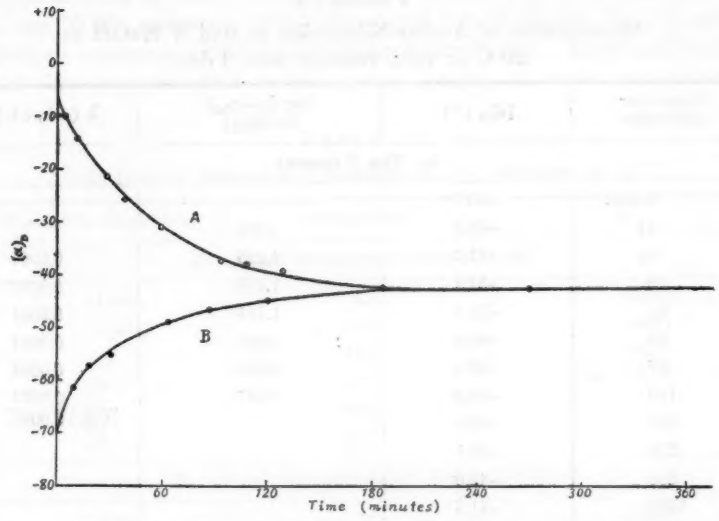

Fig. 1. Showing mutarotation of the α (A) and β (B) isomers of aniline-N-lactoside in 0.01 *N* NaOH (c 2.0) at 20°C.

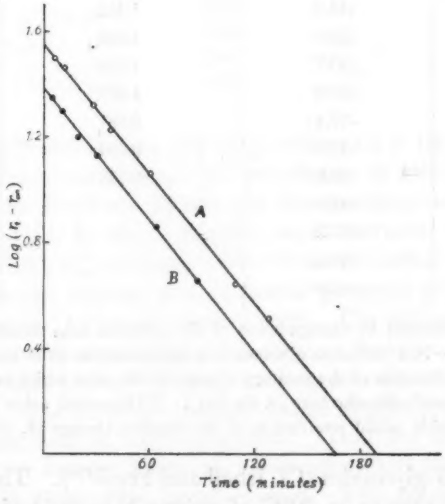

Fig. 2. $Log(r_t - r_\infty)$ (r signifies $[\alpha]_D$) of 2% solutions of the α (A) and β (B) isomers of aniline-N-lactoside in 0.01 *N* NaOH at 20°C, expressed as a function of time. Rotation tube 1 dm.

TABLE I

Mutarotation of Aniline-N-lactoside in 0.01 *N* NaOH at 20°C (c 2.0; rotation tube 1 dm.)

Time after dissolution	$[\alpha]_D$ (°)	$\log \frac{(r_t - r_\infty)}{(r = [\alpha]_D)}$	k (=k_1+k_2)
A. The β isomer			
0 min.	-65.7*		
4½	-64.6	1.354	
9½	-61.7	1.295	0.0084
18	-57.3	1.185	0.0106
31	-55.3	1.124	0.0081
64	-49.0	0.845	0.0083
87	-46.5	0.653	0.0083
120	-44.8	0.447	0.0077
180	-42.0		Avg.: 0.0085
270	-42.1		
360	-42.0		
1480	-41.3		
B. The α isomer			
0 min.	- 7.3†		
6	-10.4	1.502	0.0087
11	-14.2	1.462	0.0083
28	-21.4	1.318	0.0085
38	-25.7	1.218	0.0081
60	-30.8	1.057	0.0093
93	-37.4	0.681	0.0084
108	-37.9	0.634	0.0080
129	-39.0	0.505	Avg.: 0.0085
186	-42.3		
270	-42.2		
360	-42.2		
1480	-41.9		

*, † Values obtained by extrapolation of the observed $[\alpha]_D$ values. * The actual value might be −70.2° which is obtained in a similar manner from the rotations at pH 9.6, because acceleration of the rotatory change at the start which occurs remarkably at pH 9.6, is conceivable also here (S. the text). † The actual value might be −9.2° because of probable initial retardation of the rotation change (S. the text).

verse in case of N-glycosides (Cf. Isbell and Frush[10]). The writer followed up the rotatory chnage at 20°C of aniline-N-lactoside in buffer solutions at pH 5.6, 7.0 or 9.6, estimating simultaneously the liberated aniline in solutions, composed similar to those above and stood at the same tempera-

ture, from time to time. 84.3% aniline became free in 30 hours at pH 5.6, and 59.3% aniline in 100 hours at pH 7.0 (Table II, Fig. 3) and no hydrolysis occurred at least within 24 hours at pH 9.6. Isbell and Frush[10] found that glycosylamine is split pretty quickly in weak acid solutions

TABLE II

Hydrolysis of Aniline-N-β-lactoside in 2% solutions at 20°C

Time after dissolution	hydrolysis (%)	k_h
A. At pH 5.6 (acetate buffer 1/5 *M*)		
11 min.	4.1	0.0016
$1\frac{7}{10}$ hrs.	22.0	0.0011
$4\frac{1}{2}$	45.2	0.0010
10	66.5	Avg.: 0.0012
30	84.3	
33	84.9	
B. At pH 7.0 (phosphate buffer 1/15 *M*)		
1 hr.	2.0	0.00015
2	4.5	0.00016
$3\frac{1}{2}$	6.5	0.00014
6	11.8	0.00015
22	32.3	Avg.: 0.00015
42	44.2	
84	55.9	
100	59.3	

(pH 3–7) but only slowly in a strongly acidic (2.5 *N* HCl). Contrary to glycosylamine, the lactoside was cleft completely in a few minutes in 2 *N* HCl. In Figs. 4A & 4B are plotted the mutarotations with the figures in Tables III and IV. In the β isomer, rapid increase of dextrorotation occurred at pH 5.6, $[\alpha]_D^{20}$ reaching $+25.7°$ from the initial value of $-70.2°$ in 25 hours, but the velocity was so slowed down at pH 7.0 that the increase of $[\alpha]_D^{20}$ to $+11.0°$ took even 4 days. On the other hand, $[\alpha]_D^{20}$ curve of the α isomer at pH 5.6 fell swiftly at first to $-32°$ from $-9.2°$ and thereupon made a sharp bend upwards to join the corresponding curve of the β isomer. The curve of rotation change at pH 7.0 followed a similar but slower course with a very blunt turn. That the decrease of dextrorotation of the α isomer is due to $\alpha\beta$-isomerization and the increase to hydrolysis within these pHs is self-evident. The consistent increase of dextrorotation of the β isomer must be a combined result of those structural changes.

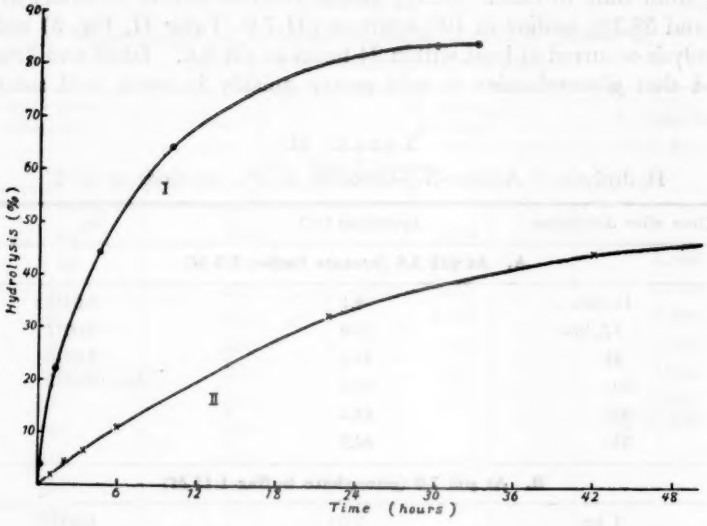

Fig. 3. Progress of hydrolysis of aniline-N-β-lactoside in 2% solutions in acetate buffer (1/5 M) pH 5.6 (I) and phosphate buffer (1/15 M) pH 7.0 (II) at 20°C, expressed as a function of time.

Hydrolysis proceeded with velocity constant k_h of 0.0012 at pH 5.6 and with that of 0.00015 at pH 7.0, as derived from the amounts of aniline liberated at the initial stage (11th to 270th minute at pH 5.6, and 60th to 360th minute at pH 7.0) by equation

$$k_h=\frac{1}{t}\log\frac{\text{Total aniline}}{\text{Total aniline}-\text{Free aniline at } t} \quad \text{.....(Eq. a)}$$

The constant of the velocity at pH 5.6 can be also calculated out from the rotation change at the early stage of the period where the α and β anomers are in equilibrium (37th to 330th minute after dissolution) by equation

$$k_h=\frac{1}{t_2-t_1}\log\frac{r_{t1}-r_\infty}{r_{t2}-r_\infty} \quad \text{.....(Eq. b)},$$

where r_∞ denotes the rotation after assumed complete hydrolysis, namely, +43.9°. This manner of calculation can not be applied for the constant of hydrolysis at pH 7.0 because the mutarotations of the both isomers take even 7 hours until their courses join together and moreover the inverse reaction is remarkable.

If, in Eq. b, k_h is substituted with 0.0012 or 0.00015, r_{t2} with the rotation at $\alpha\beta$ equilibrium, for instance, −32.6° at the 37th minute on the curves at pH 5.6 or −30.0° at the 450th minute on the curve at pH

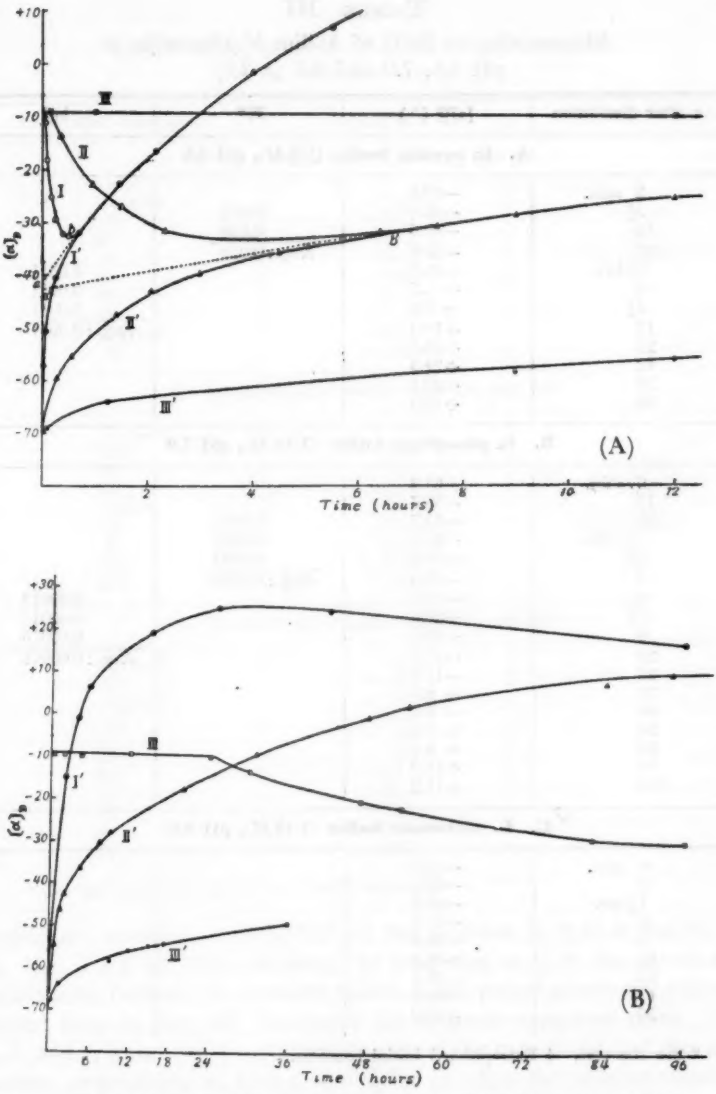

Fig. 4 (A & B). Mutarotation of the α and β isomers of aniline-N-lactoside in 2% solutions in acetate buffer (1/5 *M*) pH 5.6 (I & I'), phosphate buffer (1/15 *M*) pH 7.0 (II & II') and carbonate buffer (1/10 *M*) pH 9.6 (III & III') at 20°C, expressed as a function of time. Regarding the dotted lines a–b and a'–b', see the text.

TABLE III

Mutarotation at 20°C of Aniline-N-β-lactoside at pH 5.6, 7.0 and 9.6 (c 2.0)

Time after dissolution	$[\alpha]_D^{20}$ (°)	K*	k_h*
A. In acetate buffer (1/5 *M*), pH 5.6			
2 min.	−57.3		
$5\frac{1}{2}$	−50.9	0.052	
15	−40.6	0.058	
37	−32.6	Avg.: 0.055	
$2\frac{1}{6}$ hrs.	−16.5		0.0011
4	− 1.7		0.0011
$5\frac{1}{2}$	+ 6.8		0.0011
15	+19.2		Avg.: 0.0011
25	+25.7		
40	+24.5		
50	+23.1		
96	+18.0		
B. In phosphate buffer (1/15 *M*), pH 7.0			
6 min.	−64.9		
17	−59.7		
35	−55.7	0.0053	
$1\frac{5}{16}$ hrs.	−47.6	0.0056	
$2\frac{1}{16}$	−43.3	0.0055	
3	−39.9	Avg.: 0.0055	
$4\frac{1}{2}$	−35.5		0.00013
$7\frac{1}{2}$	−30.0		0.00011
9	−28.0		0.00010
20	−17.2		Avg.: 0.00011
26	−11.9		
31	− 8.3		
48	− 0.7		
54	+ 2.2		
84	+ 8.1		
94	+11.0		
124	+11.2		
C. In carbonate buffer (1/10 *M*), pH 9.6			
0 min.	−70.2†		
5	−69.7		
$1\frac{1}{4}$ hrs.	−64.0		
2	−62.7		
9	−58.8		
12	−56.3		
15	−55.1		
$17\frac{1}{2}$	−54.1		
36	−49.9		

* See the text. † Extrapolated initial rotation.

7.0, r_∞ with +43.9°, t_2 with 37 or 450 minutes and t_1 with 0 minute in the two cases, r_{t1} values of −41.7° and −42.4° are given which are rotations at hypothetical equilibrium of the α and β anomers without hydrolysis at pH 5.6 and 7.0 respectively. These values nearly coincide with the

TABLE IV

Mutarotation at 20°C of Aniline-N-α-lactoside at pH 5.6, 7.0 and 9.6 (c 2.0)

Time after dissolution	$[\alpha]_D^{20}$ (°)	K*
A. In acetate buffer (1/5 *M*), pH 5.6		
3 min.	−11.1	
5½	−18.3	0.055
10	−25.5	0.051
15	−29.5	Avg.: 0.053
23	−32.1	
29	−32.6	
40	−31.6	
1 9/10 hrs.	−22.9	
4½	+ 2.0	
B. In phosphate buffer (1/15 *M*), pH 7.0		
7½ min.	− 9.1	
20½	−13.8	
36	−19.2	0.0056
55	−23.0	0.0060
1½ hrs.	−24.2	0.0057
2½	−31.9	Avg.: 0.0058
4	−33.1	
6½	−31.7	
16	−20.9	
C. In carbonate buffer (1/10 *M*), pH 9.6		
0 min.	− 9.2†	
5	− 9.2	
1 1/10 hrs.	− 9.0	
4½	−10.1	
12	− 9.9	
24	−10.8	
30	−13.7	
47	−20.1	
53	−21.8	
82	−27.5	
96	−28.7	

* See the text. † Extrapolated initial rotation.

equilibrium rotation $-42.1\pm0.1°$ of the solution in 0.01 *N* NaOH (See Fig. 1). They are also obtained by extension to t_0 of the curves at $\alpha\beta$ equilibrium, because the rotation values at the points where the extensions (dotted lines in Fig. 4A) interesect the ordinate represent them. Next, let R_t and r_t stand for the rotation on one of the extensions and the actual rotation respectively at time t, and d_t for (r_t-R_t), the relative velocity of occurrence of the α and β isomers may be expressed by equation

$$K=\frac{1}{t_2-t_1}\log\frac{d_{t1}}{d_{t2}},$$

where K is the velocity constant of the interconversion of the both anomers.

Average of K values of the α and β isomers at pH 5.6, calculated as above, amounts to 0.055 and that at pH 7.0 to 0.0057. Next, K equals (K_1+K_2), and $\frac{K_1}{K_2}$ the β anomer in % of an equilibrated mixture divided by the α anomer likewise expressed. Furthermore, $[\alpha]_D$'s of these isomers themselves are $-70.2°$ and $-9.2°$ respectively and those of the mixtures at the hypothetical equilibrium ($t=0$) $-41.7°$ and $-42.4°$ at pH 5.6 and 7.0 respectively. Hence, to the solution of pH 5.6 at the hypothetical equilibrium applies the equation

$$(-9.2)x+(-70.2)y=-41.7\times 100\ ,$$

where x is the percentage of α anomer and y that of β anomer.

Therefore, $x=46.7$, $y=53.3$

and $\frac{K_1}{K_2}=\frac{53.3}{46.7}$ or $K_1=\frac{53.3}{46.7}K_2$

Hence, the values of K_1 and K_2 calculated by combining the last equation with $K=K_1+K_2$ amount:

at pH 5.6, $K_1=0.030$
$K_2=0.025$
at pH 7.0, $K_1=0.0031$
$K_2=0.0026$

The ratio of the amounts of α and β anomers at any moment is obtained from $[\alpha]_D$ values of the α and β isomers and of the hypothetical mixture at t_0 and the difference between $[\alpha]_D$'s at t on the actual mutarotation curves and respective dotted lines in Fig. 4, which is equal to the difference between the $[\alpha]_D$ at t and that at equilibrium when no hydrolysis is assumed to occur, namely, $-41.7°$ or $-42.4°$. The amounts of the anomers in per cent of the total lactoside including the hydrolyzed were reckoned by employing those ratios and hydrolysis percentages, and the figures thus obtained for the case when the α isomer was maintained at those pH's are shown in Table V, in which the percentages of the anomers at pH 9.6 and in 0.01 *N* NaOH are also given. The amount of the β anomer at pH 7.0 was larger than those at pH's 5.6 and 9.6 after 3 hours from preparation of the solutions.

At pH 9.6, aniline-N-α-lactoside differed somewhat from the β isomer in behaviour of rotation change. The latter showed slow increase of $[\alpha]_D$ from the commencement up to $-49.9°$ in 36 hours, which is ascribed to simple $\alpha\beta$-isomerization because of no occurrence of hydrolysis. In the α-lactoside, however, the rotation remained constant for the first 24 hours, followed by gradual decrease of the dextrorotation due to the change of this anomer into the other. In connection with the initial constancy of the

TABLE V

Amounts of the two Anomers of Aniline-N-lactoside, as expressed in per cent of the Total Lactoside, at Various Time after Dissolution of the α-Lactoside (c 2.0) in Solvents of Various pH's

The solutions were maintained at 20°C.

pH of the solutions	Anomer	Time in hrs.							
		0	1	2	4	10	20	36	at equil.
5.6 (Solv. acetate buffer)	α	100.0	37.1	33.1	26.7	16.2	9.7	6.9 (eq.)	6.9
	β	0.0	43.2	38.9	31.3	18.9	11.3	8.0	8.0
7.0 (Solv. phosphate buffer)	α	100.0	73.6	59.8	42.5	36.7	32.2	26.9	18.2 (100 hrs. after dis.)
	β	0.0	24.1	36.2	49.8	44.3	37.8	31.6	21.6
9.6 (Solv. carbonate buffer)	α	100.0	100.0	100.0	100.0	100.0	100.0	79.3	no eq. even after 96 hrs.
	β	0.0	0.0	0.0	0.0	0.0	0.0	20.9	
ca. 12.5 (Solv. 0.01 *N* NaOH)	α	100.0	64.3	51.7	46.0 (eq.)				46.0
	β	0.0	35.7	48.3	54.0				54.0

rotation may be mentioned the article of Worley & Andrews[11], who observed that D-glucose and D-galactose don't mutarotate according to the monomolecular equation at 0°C undergoing retardation or acceleration of the change at the start. They tried to explain the fact by assuming the accumulation of the intermediate substance at the initial stage. Whether the interpretation may hold true or not, the constant rotation of aniline-N-α-lactoside mentioned above is a phenomenon possible to occur at pH's where the mutarotation velocity of the lactoside is comparable with that of galactose at the low temperature. The cause of the adjoining gradual decrease of dextrorotation must be mere anomerization, because of no occurrence of hydrolysis as above. For a similar reason, acceleration of the mutarotation at the very beginning is accepted for the β-lactoside (See also the note of Table I).

Absorption spectra. Ultraviolet absorption was examined in a Hitachi EPB-U type spectrophotometer. The spectra of the α- and β-lactoside in 0.01 *N* NaOH were identical with each other with a maximum at the wave length 237.5 mμ and were not altered by $\alpha\beta$-isomerization on standing. The spectra here resemble that of aniline closely in shape but is shifted towards longer waves with increase of extinction (batho- and hyperchromic effect of the glycosidic sugar) (Table VI, Fig. 5). The α-lactoside in the acetate buffer pH 5.6 showed also the same spectrum as above im-

mediately after dissolution, which, however, became nearly identical with that of aniline (λ_{max1} 230 mμ, ε_{max1} 5996; λ_{max2} 280 mμ, ε_{max2} 944; λ_{min} 257.5 mμ, ε_{min} 281) in 25 hours, approaching to the latter by and by with time due to hydrolysis of the substance (Table VI).

TABLE VI

Ultraviolet Absorption of Aniline-N-lactoside

2% solutions of the α- and β-lactoside in various solvents were stood at 20°C and examined at various time spectrophotometrically with portions 1000-fold diluted with the respective solvents. Figures express wave lengths in mμ. In parentheses are given the respective molecular extinctions (ε)

Anome		Time after dissolution in hrs.			
		0	2	10	25
A. Solvent 0.01 *N* NaOH					
β-Anomer	λ_{max1}	237.5 (12940)	237.5 (12971)	237.5 (12916)	
	λ_{max2}	285.0 (1593)	285.0 (1599)	285.0 (1591)	
	λ_{min}	262.5 (815)	262.5 (826)	262.5 (808)	
α-Anomer	λ_{max1}	237.5 (11408)	237.5 (11398)	237.5 (11388)	
	λ_{max2}	285.0 (1361)	285.0 (1359)	285.0 (1356)	
	λ_{min}	262.5 (653)	262.5 (653)	262.5 (651)	
B. Solvent acetate buffer (1/5 *M*), pH 5.6					
α-Anomer	λ_{max1}	237.5 (11112)			232.0 (6380)
	λ_{max2}	285.0 (1190)			280.0 (968)
	λ_{min}	262.5 (350)			258.0 (286)

EXPERIMENTAL

Preparation of aniline-N-β-lactoside (*I*). 2.6 g. of lactose and 1 cc. of aniline(redistilled) were taken up in 1.5 cc. of water and shaken vigorously in boiling water. The mixture became homogeneous and light brown after 20 minutes and solidified after 30 minutes. Then it was stood in a refrigerator overnight, brought onto a suction filter and washed with aqueous methanol ($CH_3OH : H_2O = 1 : 1$ by volume) and then with abs. methanol. Yield 2.5 g. (80% of theory), F.P. 187–190°. Recrystallized three times from the aqueous methanol, it was obtained in thin needles with F.P. of 199–201°. $[\alpha]_D^{20}$: −82.1°

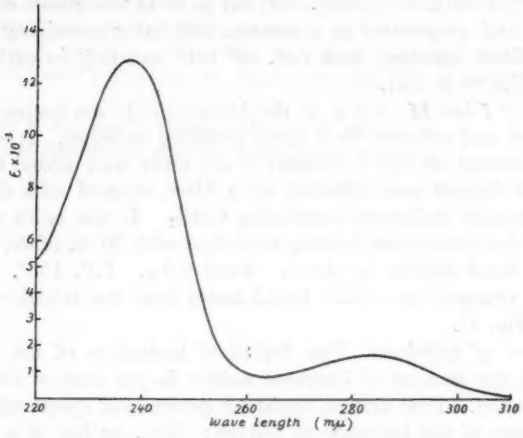

Fig. 5. Ultraviolet absorption spectrum of aniline-N-β-lactoside in 0.01 *N* NaOH (at 20°C).

(c 0.5) in dry pyridine; −70.2°[12] (c 2.0) in carbonate buffer (0.1 *N*) of pH 9.6. The product was difficultly soluble in pyridine, methanol and dioxane and insoluble in ethanol, acetone and ether.

N (micro Kjeldahl)

$C_{18}O_{10}H_{27}N$: Calc. 3.36%; found 3.33%.

Preparation of aniline-N-α-lactoside (***II***). 2 g. of lactose and 0.6 cc. of aniline were refluxed, avoiding moisture, with 100 cc. of dry methanol for 15 hours. A small insoluble part of lactose was filtered off and to the clear filtrate were added 8 volumes of dry ether. Crystals were deposited in thin needles, which were filtered off, washed with 3 changes of dry ether and dried in vacuo over $CaCl_2$. The substance obtained melted at 158–159°. Yield here 1.2 g. (49% of theory). It was recrystallized twice without elevation of the melting point. The product was hygroscopic. $[\alpha]_D^{20}$: −13.5° (c 0.5) in dry pyridine; −9.2°[12] (c 2.0) in carbonate buffer of pH 9.6. Its solubilities in organic solvents resembled those of the β-isomer.

N (micro Kjeldahl): Found 3.37%.

From the mother fluid of the crude product, placed in a refrigerator, came out gradually large needles amounting in total to 0.2 g., which showed F.P. of 189° and $[\alpha]_D^{20}$ in carbonate buffer pH 9.6 of −65.8°[12] (c 1.5), proving the crude β anomer.

Conversion of ***II*** *into* ***I***. 1) The α anomer (**II**) was stood in air for 2 months. The crystals, which became tinted light brown, had F.P. of 185–187° and $[\alpha]_D^{14}$ in carbonate buffer pH 9.6 of −62.7°[12] (c 1.0). 2) 0.1 g. of **II** was dissolved in 1 cc. of 50% methanol by warming and allowed to cool, whereby needles came out. Yield 0.08 g. F.P. was 195–197° and $[\alpha]_D^{14}$ in carbonate

buffer of pH 9.6—67.2°[12] (c 1.0). 3) 0.1 g. of **II** was dissolved in 50 cc. of dry methanol and evaporated in a vacuum desiccator containing $CaCl_2$. The crystalline residue (needles) had F.P. of 191° and $[\alpha]_D^{16}$ in carbonate buffer pH 9.6 of −67.3°[12] (c 1.2).

*Conversion of **I** into **II**.* 0.6 g. of the β-lactoside (**I**) was suspended in 100 cc. of dry methanol and refluxed for 6 hours avoiding moisture. A small insoluble part of **I** was filtered off and 7 volumes of dry ether were added to the filtrate. The crystalline deposit was collected on a filter, washed with dry ether and placed in a vacuum desiccator containing $CaCl_2$. It was twice reprecipitated by the aid of dry ether after heating each time with 90 cc. of dry methanol for 5 hours, and dried similar to above. Yield 0.3 g. F.P. 158°. $[\alpha]_D^{20}$ of it in 0.01 *N* NaOH changed to −42.2° in 3.5 hours from the initial −9.2° (c 2.0) (Comp. with Fig. 1).

Measurement of hydrolysis. The degree of hydrolysis of the lactoside was expressed with the amount of liberated aniline in per cent of total aniline including the bound. Free aniline could be determined spectrophotometrically without cleavage of the lactoside as follows: 3 cc. or less of a solution containing 0.06–24 mg. free aniline is pipetted out of a lactoside solution into a stoppered (ground joint) flask, made up to 3 cc. with water, when necessary, and quickly alkalized with 0.15 cc. of 10% NaOH, followed by shaking for just 1 minute. The mixture is then added to with 0.015 cc. of the sat. $CaOCl_2$ solution (freshly prepared), shaken again accurately for 1 minute and stood for 1 hour at room temperature (20°C) to measure thereupon the extinction at the wave length 365 mμ. The amount of free aniline is read from the standard curve.

Summary

1. The α and β isomer of aniline-N-lactoside were prepared.

2. The mutarotation of them in buffer solutions of pH 5.6 and of pH 7.0 is combined result of hydrolysis and anomerization, and that in a buffer pH 9.6 and 0.01 *N* NaOH is caused by mere anomerization.

3. The progress of anomerization and hydrolysis is analyzed mathematically.

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Synthesis of Artificial Group A and B Substances*

By

Hajime Masamune, Sen-itiroh Hakomori, Haruo Numabe,
(正宗 一) (箱守 仙一郎) (沼部 治夫)
Ziroh Akama and **Sigetosi Kamiyama**
(赤間 二郎) (加美山 茂利)

From the Medico-chemical Institute, Tohoku University, Sendai

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Regarding blood group substances from various kinds of body fluid and visceral organ, Masamune[1] one of us claimed in 1947 that certain hexosamine-galactosides or disaccharides of structures related to them act as the unit determinant group to which α- or β-agglutinin is adapted complementarily. He also announced,[2),2a)] being based on the phenomenon of interconversion in vitro of Group A and B in a group lipoid, that the foremost structural difference existing between Group A and B substances exists in which mode of union, α- or β-glycosidic, the carbohydrate moity, which constitutes the determinant group, is linked with aglycon portion in the molecule. According to him, the aglycon most probably shields the right or wrong side of the plain carbohydrate molecule or one of the cut ends of the coiled carbohydrate molecule, laying the other side or the other cut end bare to combine with the agglutinin. Our later structural study[3)4)4a)] indicated that blood group lipoids are N-glycosides of polysaccharides consisting of chondrosamine, galactose and mannose or of hexosamines and galactose, and that the aglycon is a lipid-polypeptide complex whose glutamyl residue participates with its amino group in linkage with the polysaccharide. On the other hand Yamakawa[5)] discovered, apart from our studies, remarkable group potency in "globosides"[6)] which comprise an acylsphingosine and a carbohydrate made up of 1 molecule of N-acetylchondrosamine, 3 molecules of galactose and a trace of glucose. After these considerations, it appeared to us that group active substances might be prepared only by *adequately* fixing N-glycosidically a hexosamine-galactoside to different amphipatic structures, that form large micelles, such as phosphatidylpeptide, sphingosine and proteinic materials. Hence we synthesized two series of N-glycosides of hexosamine-4-β-galactoside and of its dimer, employing firstly various phosphatidyl-

* 175th report of "Biochemical Studies on Carbohydrates"; 19th report of "Chemistry and Biology of Lipids"; 10th report of "On Proteins and Amino Acids."

peptides and secondly sphingosine and a 1-O-acylsphingosine, and found most of the former N-glycosides Group B potent and most of the latter Group A potent. We further witnessed that one of the latter group of compounds partially changed into a Group B substance on standing in physiol. saline in harmony with mutarotation from right to left, and also that one of the former neither mutarotated nor showed group alteration. This correlation of group conversion and mutarotation suggests that the carbohydrate moiety is generally bound α-glycosidically in Group A substances and β-glycosidically in Group B substances.

Experimental

Materials and Synthesis of Typical Group-active Substances

1) (N-Acetyl)-glucosamine-4-β-galactoside and (N-acetyl)-glucosamine-4-β-galactosido-2-β-(N-acetyl)-glucosaminido-4-β-galactoside[7]

Preparation procedure. Yosizawa's procedure[7a] for separating N-acetylglucosamine-4-β-galactoside was modified. Namely, 3 g. of a product at the third stage of his procedure for preparing the group mucopolysaccharide (the main part) from pig stomach mucus were subjected to acetolysis by treatment with 25 cc. of acetic anhydride and 3.5 cc. of 99.5% sulfuric acid at 15–20°C for 2 days and subsequently at 52–53°C for 12 hours. The centrifuged supernatant was poured into 120 cc. of ice-water containing 20 g. of crystalline sodium acetate under agitation, whereby a red-brown oily deposit appeared. After addition of Na_2CO_3 and $NaHCO_3$ powders to neutral, whereby the oily deposit changed thick-pasty, the solution containing the deposit was extracted with two 70 cc. portions of chloroform. The chloroform solutions were combined, washed with three 100 cc. portions of water and dried with 10 g. of $CaCl_2$. They were then distilled in vacuo (30°C) to a syrup, and after taking up in 5 cc. of dry methanol, placed in a refrigerator overnight. The precipitate was filtered off with suction and washed with dry methanol, and the filtrate and washing were distilled together under diminished pressure and dried in vacuo over H_2SO_4.

Next, the product (2.5 g.) was shaken with 113 cc. of 0.2 *N* KOH for 4 hours (15–20°C) and the dark brown solution was neutralized with dilute sulfuric acid and distilled under diminished pressure to a syrup. This was further distilled with changes of dry methanol to eliminate water as far as possible, then refluxed with 50 cc. of dry methanol for 5 minutes and filtered hot with suction. The filtrate was again condensed to a syrup, boiled with 50 cc. of ethanol and filtered similar to above. The ethanol-insoluble part on the filter was washed with three 10 cc. portions of hot ethanol and dried—Crude product of the tetrasaccharide. This material weighing 250 mg. was dissolved in 3 cc. of water and added to with 40 cc. of methanol. Some flocculent deposit was rejected by centrifugation and the supernatant was precipitated with 200 cc. of ether and dried. Followingly, it was dissolved in 100 volumes of water and passed through columns of cation exchange (Amberite IR-120) and anion ex-

change (Amberite IR-4B) resins (10 mm. × 27 cm.) three times each, followed by distillation. The still-residue was washed with an ethanol-ether mixture (2:1 by volume) and dried to a constant weight in vacuo over H_2SO_4—(N-Acetyl)-glucosamine-4-β-galactosido-2-β-(N-acetyl)-glucosaminido-4-β-galactoside. Yield 180 mg. It was a very hygroscopic, brownish powder.

On the other hand, the filtrate of the crude tetrasaccharide and washings were cooled together and about 50 cc. of ethanol were added until no more precipitate occurred. The centrifuged supernatant was distilled to a syrup, and exhausted with 50 cc. of cold abs. ethanol. The solution here was inspissated as far as possible. The non-colored powder obtained was taken up in 20 cc. of butanol, and a flocculent precipitate occurring on additional addition of butanol to 200 cc. was centrifuged and dissolved in 30 cc. of 70% ethanol. The insoluble was discarded and the solution was distilled in vacuo. The syrupy residue here was dissolved in 10 cc. of 70% ehtanol and added to with 50 cc. of abs. ethanol and 20 cc. of ether. Some deposit was removed by means of a centrifuge, and the supernatant was precipitated by the aid of 500 cc. of ether and dried. The substance given was treated with the resins, recovered and dried as above. 410 mg. of a non-colored powder were yielded—N-Acetyl-glucosamine-4-β-galactoside.

Properties. a) N-Acetylglucosamine-4-β-galactoside. It melted under decomposition at 174–179°C. Indirect-Osaki-Turumi and Molisch reactions positive, direct-Osaki-Turumi negative. Analysis: N (micro Kjeldahl) 3.62% (Calc. 3.65%); glucosamine (Blix) 46.6% (Calc. 46.7%); acetyl (a modification of Friedrich, Rapoport and Sternberg method) 11.2% (Calc. 11.2%); iodine use as glucose (Macleod & Robison) 48.2% (Calc. 47.0%), ash (Pregl without use of H_2SO_4) 0.67%. Rotation: $[\alpha]_D^*$ in water +65.2° (4 min. after dissolution)→+58.1°. Sugar-paper-chromatographically, it gave only one spot by either of a basic (a butanol-pyridine-water (5:3:2 by volume) mixture) and an acidic (a *n*-butylacetate-acetic acid-water (4:1:2 by volume) mixture) solvent, and its hydrolysate two spots corresponding to glucosamine and galactose by the former solvent (Fig. 1).

The chromatographic procedure of the monosaccharide components. 5 mg. of the substance dissolved in 0.5 cc. of 1 *N* HCl were heated in an ampoule at 100°C for 5 hours, and freed from HCl by repeated condensations with water in a vacuum desiccator containing solid caustic soda, without evaporating to dryness until a condensate of pH 2.0 was given. It was freed from the insoluble matter in a centrifuge, the evaporated dry residue was dissolved in 0.1 cc. of water and neutralized with NaOH and 0.02 cc. was sampled. Irrigation descending. Solvent butanol-pyridine-water (5:3:2 by volume). Temperature and duration of test 20–22°C and 24 hours respectively.

It was found inactive with respect to all of groups A, B and O (Normal goat- and anti-O saliva chicken-serum were used for Group O assay) even at a dilution of 1:50. The substance thus proved identical with the preparation of Yosizawa[7a].

b) (N-Acetyl)-glucosamine-4-β-galactosido-2-β-(N-acetyl)-glucosaminido-4-β-galactoside. It was Molisch and indirect-Osaki-Turumi positive but biuret

Fig. 1. Butanol-pyridine-water chromatogram of the monosaccharide components of the disaccharide and tetrasaccharide (Regarding the procedure, see the text).
a: reference run, b: the disaccharide, c: the tetrasaccharide. 1: chondrosamine hydrochloride, 2: glucosamine hydrochloride, 3: galactose, 4: mannose, 5: rhamnose.

and direct-Osaki-Turumi negative, and analyzed: N 3.80% (Calc. 3.74%); glucosamine 47.8 % (Calc. 47.9%); acetyl 11.4% (Calc. 11.5%); iodine use as glucose 23.8% (Calc. 23.8%); ash 0.73%. The rotation was measured after treatment of the substance with activated charcoal. $[\alpha]_D^5$ in water +28.9°(4 min. after dissolution)→+24.6°. Paper chromatography of a hydrolysate effected similar to above demonstrated the presence of glucosamine and galactose but no other sugar (Fig. 1). It had no group potency like the disaccharide.

2) Phosphatidyl-(α-glutamyl)-ethanolamine-N-β-(N-acetyl)-glucosaminido-4-β-galactosido-2-β-(N-acetyl)-glucosaminido-4-β-galactoside (I)

Phosphatidylethanolamine. The phosphatidylethanolamine employed as a material was isolated according to Folch[8] from a 3 g. portion of a batch of the cephalin fraction of brains from Group O persons. Its emulsion was dialyzed against running water for 1 week, and precipitated with a large excess of acetone. The deposit was washed with the same solvent repeatedly until it took fluffy appearance, and dried over P_2O_5 in vacuo to a nearly constant weight. Yield 230 mg. N 1.79%; P (modified Plimmer) 3.70%; Molisch negative. (Calc. for the stearyl-oleyl $C_{41}H_{80}O_8NP$: N 1.88%, P 4.16%).

N-Carbobenzoxyglutamyl-(phosphatidyl)-ethanolamine. 200 mg. of phosphatidylethanolamine above were dissolved in 5 cc. of dry chloroform[9] and a solution of 70 mg. of carbobenzoxyglutamic anhydride[10] in 2 cc. of the same solvent

was added, and the mixture was refluxed for 30 minutes, followed by standing overnight at room temperature. Then, the chloroform was evaporated off, and the remainder was dissolved in 30 cc. of dry ether. Some crystalline precipitate (carbobenzoxyglutamic acid) that occurred on standing for a short time was filtered off. The filtrate and washings were distilled in vacuo as far as possible and extracted twice with 10 cc. portions of warm acetone, and the insoluble (probable phosphatidylethanolamine which had not reacted) was rejected. The combined acetone solutions were chilled at −10° and the yellowish precipitate was quickly centrifuged (30 sec. at 3000 r.p.m.), washed with chilled acetone and dried in vacuo over H_2SO_4. Yield 110 mg. Since the substance obtained here showed slight positive ninhydrin reaction,[11] it was purified by many reprecipitations from acetone, when it was almost non-colored and ninhydrin-negative. Yield 90 mg. N 2.85%; P 3.15% (Calc. for $C_{54}H_{93}O_{13}N_2P$: N 2.77%; P 3.07%).

Splitting off of N-carbobenzoxy group. The balance (70 mg.) of carbobenzoxyglutamyl-(phosphatidyl)-ethanolamine was dissolved in a mixture of 15 cc. of ethanol and 5 cc. of glacial acetic acid and hydrogenated by the aid of Pd-black of Willstätter & Waldschmidt-Leitz[12] freshly prepared. About 80 cc.[13] of hydrogen were consumed in 3 hours. After the catalyst was centrifuged off, the transparent brownish supernatant was distilled in vacuo. The still-residue was extracted with warm acetone, and a bulky precipitate occurring when the solution was chilled at 0° was centrifuged and reprecipitated. The product with yellowish hue exhibited marked ninhydrin reaction. Yield 55 mg. N 3.12% (Calc. for $C_{46}H_{87}O_{13}N_2P$ 3.07%).

Condensation with the tetrasaccharide. 40 mg. of the phosphatidyl-(glutamyl)-ethanolamine and 15 mg. of the tetrasaccharide above were taken up in a mixture of 3 cc. of absolute methanol and 0.01 cc. of glacial acetic acid and refluxed, avoiding moisture, in a glycerol bath at about 80–90° for 12 hours, whereby the tetrasaccharide went gradually into solution. Cooled. A portion still remaining undissolved was filtered off. The filtrate and washings were distilled in vacuo to about 0.5 cc. and a part of the polysaccharide, which had not reacted but was in solution, was carried down by addition of 3 cc. of chloroform and filtered off. The filtrate was condensed to a syrup by distillation under reduced pressure and completely dried over P_2O_5 in a vacuum desiccator. It was then stirred up in 3 cc. of dry ether and the opalescent solution given was cooled at 0°. The deposit here was quickly centrifuged and dried as above. Yield 20 mg. This end product was very soluble in chloroform, moderately soluble in methanol, less soluble in alcohol and benzene, and insoluble in acetone, ether (dry) and petrol ether. N 3.3% (Calc. for $C_{74}H_{134}O_{31}N_4P$ 3.48%).

3) Hydrophosphatidyl-(γ-carbomethoxy-α-aminobutyryl-glycyl-glycyl)-ethanolamine-N-β-(N-acetyl)-glucosaminido-4-β-galactosido-2-β-(N-acetyl)-glucosaminido-4-β-galactoside (**II**)

Catalytic hydrogenation of phosphatidylethanolamine. 350 mg. of phosphatidylethanolamine prepared as in 2) were dissolved in a mixture of 30 cc. of ether and 8 cc. of glacial acetic acid and shaken with hydrogen under atmospheric

pressure using 0.5 g. of active Raney nickel[14] as a catalyst, whereby turbidity appeared. 150 cc. of hydrogen were absorbed in 3.5 hours. The fluid was clarified by addition of chloroform and then the catalyst was centrifuged off. The supernatant was distilled under reduced pressure to syrupy consistency, and after precipitation with a large excess of methanol, washed twice with acetone and dried in a desiccator ($CaCl_2$). The fluffy powder obtained was soluble readily in chloroform and sparingly in ether and glacial acetic acid, but not in methanol, ethanol and acetone. Yield 320 mg.

Acylation of the amino group of hydrophosphatidylethanolamine with carbobenzoxyglycylglycine chloride. This acylation was carried out by Yamashita and Yashiro's method[15] in stead of Schotten and Baumann's, because browning of the reaction mixture happens in the alkaline medium if the latter method is applied. 5.2 g. of crude carbobenzoxyglycylglycine were prepared after Bergmann & Zervas[10] with 4.5 g. of glycinanhydride and 7.6 g. of carbobenzoxychloride. It was recrystallized from methanol and 4.8 g. of a pure product with melting point of 171° were given. 0.1 g. of it was dissolved in 2 cc. of chloroform, and warmed gently together with 0.5 cc. of purified thionylchloride[16] for 30 minutes at 60°C, disregarding slight browning. The reaction mixture was then distilled in vacuo until the offensive smell of thionylchloride disappeared (It took several hours), taken up in 5 cc. of benzene and cooled. In the next, 300 mg. of hydrophosphatidylethanolamine were dissolved in 10 cc. of glacial acetic acid containing 40 mg. of anhydrous sodium acetate, added to with the solution of carbobenzoxyglycylglycine chloride obtained above, under cooling in water, and stood at room temperature protecting from moisture. Within 1 hour NaCl crystals began to precipitate. After 24 hours the deposit was centrifuged off, and the clear supernatant was distilled at below 30°C. The still-residue was dissolved in 10 cc. of chloroform, and the solution was washed twice with water, dried with $CaCl_2$ and distilled in vacuo. To the remainder here was added hot alcohol, some insoluble matter was filtered off while hot, and the precipitate occurring on standing the solution at 0°C was separated in a centrifuge. It was reprecipitated from 10 cc. portions of alcohol and a yellowish-brownish, ninhydrin-negative powder was obtained. This was dissolved in benzene and treated with charcoal, but no perfect decolorization was achieved. Hence the solution was distilled, and the still-residue was dissolved in hot alcohol and after addition of acetone, stood at −10°C. A bulky yellowish-tinged precipitate was quickly centrifuged cold and dried. Yield 240 mg. N 4.1%, P 3.05% (Calc. for $C_{53}H_{94}O_{12}N_3P$: N 4.2%, P 3.1%).

Decarbobenzoxylation. Carbobenzoxyglycylglycyl-(hydrophosphatidyl)-ethanolamine weighing 220 mg. was dissolved in a mixture of 10 cc. of glacial acetic acid and 10 cc. of ether, and shaken with hydrogen in the presence of 0.2 g. of Pd-black of Willstätter & Waldschmidt-Leitz. 48 cc. of hydrogen were consumed in 3 hours but no more on further shaking. The centrifuged supernatant was distilled to dryness and washed with acetone. The tan powder yielded weighed 180 mg. It gave strong ninhydrin reaction.

Further acylation with carbobenzoxyglutamic anhydride. The product above was

completely dried over P_2O_5. And 150 mg. of it and 40 mg. of carbobenzoxy-glutamic anhydride were each dissolved in 5 cc. of dry chloroform, mixed together and after addition of a few drops of absolute pyridine, heated over an oven under reflux for 1 hour, followed by standing overnight at room temperature. The mixture was washed with 1 *N* HCl, 1 *N* Na_2CO_3 and water in turn, dried with $CaCl_2$ and distilled in vacuo to a sticky syrup, which became fluffy when kneaded with several changes of cold acetone. It was next taken up in a hot alcohol-acetone (1:1 by volume) mixture, the insoluble part was removed and the solution was precipitated by cooling at 0°C, spun while cold and dried in vacuo over H_2SO_4. The product was soluble in ether, ethanol and methanol, though sparingly, but not in acetone and petrol ether. Ninhydrin negative. Yield 118 mg. N 5.38%, P 3.11% (Calc. for $C_{58}H_{102}O_{15}N_4P$: N 5.45%, P 3.11%).

Methylation with diazomethane and decarbobenzoxylation. All the balance (100 mg.) of the substance above was dissolved in 100 cc. of dry ether containing diazomethane, which had been derived from 1 g. of nitrosomethylurea,[17] and a few cc. of methanol were added as a catalyst. The mixture was stood at room temperature for 2 hours (Mild evolution of nitrogen lasted for about 1 hour) and vacuum-distilled to dryness, then, the residue was taken up in 12 cc. of a mixture of chloroform, glacial acetic acid and ether (1:1:1 by volume) and hydrogenated in the presence of 0.2 g. of Pd-black. About 50 cc. of hydrogen were used in the course of 1.5 hours. The centrifuged supernatant was distilled at low temperature to dryness, washed well with abs. alcohol and placed in a vacuum-desiccator (H_2SO_4). Ninhydrin strongly positive. Yield 95 mg. N 5.60%, P 3.20% (Calc. for $C_{51}H_{98}O_{13}N_4P$[18]: N 5.56%, P 3.17%). A hydrolysate by 6 *N* HCl gave on paper chromatograms (Solvs. 80% phenol and butanol sat. w. water-phenol sat. w. water-glacial acetic acid (5:3:1)) three ninhydrin-reacting spots corresponding to glutamic acid, glycine and ethanolamine.

Condensation with the tetrasaccharide. 50 mg. of the product and 40 mg. of the tetrasaccharide were taken up in a mixture of 3 cc. of dry chloroform and 1 cc. of absolute methanol, and 0.01 cc. of glacial acetic acid was added. The reaction mixture was boiled under reflux, avoiding moisture, on a boiling water bath for 12 hours, until most of the tetrasaccharide dissolved. A small undissolved part of the carbohydrate was filtered off, and the filtrate was distilled in vacuo to perfect dryness and exhausted with two 2 cc. portions of chloroform. The chloroform solutions were concentrated together to 1 cc., shaken well with 2 cc. of dry ether and cooled at 0°C. An almost non-colored, uniformly shaped, half-crystalline deposit occurred, which was quickly centrifuged, washed once with ether and dried in a vacuum-desiccator ($CaCl_2$). Yield 35 mg. The substance was soluble easily in chloroform and methanol, less in ethanol and glacial acetic acid and hardly in benzene, but insoluble in ether, petrol ether and acetone. N 4.90%, glucosamine 20.0% (Calc. for $C_{79}H_{144}O_{33}N_6P$: N 4.83%, glucosamine 20.52%).

4) Hydrophosphatidyl-(γ-carbomethoxy-α-aminobutyryl-glycyl-glycyl)-ethanolamine-N-β-(N-acetyl)-glucosaminido-4-β-galactoside (**III**)

N-Acetylglucosamine-4-β-galactoside was replaced for the tetrasaccharide at the last stage of preparing **II**, and 23 mg. of a product aimed at were yielded from 15 mg. of the disaccharide and 35 mg. of the other component. It showed almost the same solubilities as **II**. N 5.21%, glucosamine 12.8% (Calc. for $C_{65}H_{121}O_{23}N_5P$: N 5.10%, glucosamine 13.0%).

5) α-Glutamyl-(α,α'-dipalmitylglycero-β-phosphoryl)-ethanolamine-N-β-(N-acetyl)-glucosaminido-4-β-galactoside (**IV**)

α,α'-Dipalmitylglycyerophosphoryl-(N-carbobenzoxy)-ethanolamine. Rose[20] was followed essentially. Into a mixture of 3.06 g. of phosphorus oxychloride,[19] 25 cc. of dry pyridine and 25 cc. of chloroform (under vigorous agitation in a two-necked flask), 50 cc. of chloroform containing 11.4 g. of α,α'-dipalmitin[20] were poured in small portions, and after 30 minutes, 10 cc. of a chloroform solution containing 3.90 g. of N-carbobenzoxy-ethanolamine[21] were added under the conditions of Rose. The mixture was stirred up well and then after further addition of a mixture of 0.3 cc. of water and 4 cc. of pyridine and repeated agitation, freed from chloroform by distillation in vacuo. To the remaining solution were added 100 cc. of water, 50 g. of snow and 200 cc. of ether in sequence. The mixture here was stirred up for a few minutes and transferred to a separatory funnel to further shake energetically. Since the emulsion formed did not separate into layers on standing, it was acidified with HCl (Sometimes sodium chloride was added instead) and centrifuged in a large tube for 30 minutes (2000 r.p.m.). The upper ethereal layer was siphoned out, and the lower emulsion layer was shaken again with ether and centrifuged. The ethereal solutions were washed with water and after drying with anhydrous sodium sulfate, distilled to half the volume to place in a refrigerator (0°C) overnight. A crystalline deposit that occurred was filtered off. The filtrate was vacuum-distilled to dryness and extracted with three 100 cc. portions of warm methanol, whereby the most part dissolved and the sticky insoluble adhered to the bottom of the vessel. The methanolic solutions were united and cooled at 5° and the bulky deposit was filtered off with suction, which took a long time. The filtrate hereby was concentrated to 100 cc. and cooled at 0°C. Turbidity occurred but was only slight so that the fluid was cooled at −20° for 15 minutes and the appearing abundant white lustrous crystals were quickly separated by centrifugation (30 sec. at 4000 r.p.m.). Dissolution in methanol and precipitation by strong chilling was effected twice more and snow-white lustrous crystals given were dried in vacuo over $CaCl_2$. Yield 2.2 g. The substance melted at 44°, sintering at 43°. N 1.65%, P 3.50% (Calc. for $C_{45}H_{80}O_{10}NP$: N 1.70%, P 3.75%). 50.91 mg. of it were dissolved in 10 cc. of warm alcohol, and titrated with 0.05 *N* NaOH (Solv. 50% alcohol), taking phenolphthalein as an indicator, and 1.35 cc. of the NaOH solution were found used (Calc. 1.26 cc.).

Decarbobenzoxylation.[22] 1.8 g. of the product above were dissolved in 30 cc. of glacial acetic acid, and shaken for 2 hours together with hydrogen and 0.2 g. of freshly prepared Pd-black of Tausz & Putnocky[23] in a 300 cc. reduction flask. Hydrogen absorbed amounted to 110 cc. The solution, which became turbid, was clarified by addition of chloroform and filtered. The filtrate was

distilled in vacuo and the sticky residue was taken up in 50 cc. of hot acetone and cooled at 0°C. The voluminous flocculent precipitate hereby was quickly spun, reprecipitated likewise three times, and after washing with ether, dried in vacuo over P_2O_5. A fluffy almost non-colored powder given sintered at about 120° and melted at 168° and exhibited markedly positive ninhydrin reaction. It was insoluble in cold methanol, acetone and ether, slightly soluble in hot acetone and cold glacial acetic acid, and readily soluble in chloroform. Yield 1.2 g. N 2.09% (Calc. for $C_{87}H_{74}O_8NP$ 2.06%).

Acylation with carbobenzoxyglutamic anhydride. The substance obtained above was thoroughly dried in an Abderhalden apparatus (35°C), and 1.0 g. of it in 15 cc. of dry chloroform and 320 mg. of N-carbobenzoxyglutamic anhydride in 5 cc. of the chloroform and 1 cc. of dry pyridine were refluxed together for 30 minutes, and stood at room temperature for 10 hours. Then, the mixture was washed with several changes of 1 *N* HCl, with 5% sodium carbonate until it became almost neutral and with water in turn and dried with anhydrous sodium sulfate, followed by distillation at low temperature to dryness. The still-residue was stirred up with 15 cc. of an acetone-methanol (1 : 1 by volume) mixture and the centrifuged clear supernatant was cooled at −10°. A bulky flocculent precipitate that appeared (tinted brownish) was centrifuged, dissolved in acetone and after agitation with activated alumina, filtered. And the filtrate and washings were distilled together to dryness as above, taken up in hot methanol and centrifuged while warm to liberate from some insoluble. The supernatant was cooled at −10°, and a white half-crystalline precipitate was centrifuged, washed with ice-cold methanol and dried in vacuo over $CaCl_2$. Yield 0.8 g. It sintered at 45° and melted at 51°. N 2.4%, P 3.23% (Calc. for $C_{50}H_{87}O_{13}N_2P$: N 2.90%, P 3.34%). The water-soluble components after hydrolysis gave spots of glutamic acid and ethanolamine on a paper-chromatogram (Solv. 80% phenol) which analyzed equimolar.

Decarbobenzoxylation. Processed as above, 0.55 g. of a fluffy, almost non-colored, ninhydrin positive powder was given from 0.7 g. of the carbobenzoxy derivative. It exhibited birefringence in polarized light, and melted at 85°, having sintered at 80°. 2.08 cc. of 0.05 *N* alcoholic NaOH were used to neutralize 45 mg. of it in 10 cc. of warm ethanol (The quantity of the NaOH solution calculated 2.21 cc.).

Condensation with the disaccharide. 180 mg. of α,α'-dipalmitylglycerophosphoryl-(α-glutamyl)-ethanolamine obtained above and 80 mg. of the disaccharide were boiled with 6 cc. of an abs. ethanol-abs. methanol (1 : 1 by volume) mixture under a return condensor fitted with a $CaCl_2$-tubelet (A glycerol bath at 90–100° was used). Boiling was continued for 12 hours. The sugar dissolved entirely within the first 1 hour. The reaction mixture colored light yellow was stood in a refrigerator (0°) overnight. A voluminous crystalline deposit was given, which was centrifuged and washed with three 3 cc. portions of dry ether. It was next dissolved in 5 cc. of dry chloroform, and filtered and the filtrate was subjected to vacuum-distillation. The dry remainder was taken up in 3 cc. of the ethanol-methanol mixture by heating and cooled

slowly to room temperature. On scratching the vessel wall with a glass rod a half-crystalline deposit appeared. This was centrifuged and dried in vacuo over H_2SO_4. Yield 45 mg. Molisch strongly positive. N 3.30%, P 2.51%, glucosamine 15.0% (Calc. for $C_{55}H_{104}O_{21}N_3P$: N 3.58%, P 2.81%, glucosamine 15.26%).

6) γ-Glutamyl-(α,α′-dipalmitylglycerophosphoryl)-ethanolamine-N-β-(N-acetyl)-glucosaminido-4-β-galactoside (**V**)

γ-Phthalylglutamyl-(α,α′-dipalmitylglycerophosphoryl)-ethanolamine. In general, acylation with N-phthalylglutamic anhydride gives a γ-glutamyl derivative[24] contrary to the case with N-carbobenzoxyglutamic anhydride, where an α-glutamyl derivative is produced. Therefore, the synthesis was carried out as follows. 0.5 g. of α,α′-dipalmitylglycerophosphoryl-ethanolamine and 0.18 g. of phthalylglutamic anhydride[25] were dissolved together in 3 cc. of dry chloroform, and after addition of 0.2 cc. of dry pyridine, refluxed for 1 hour over an oven, with subsequent standing at room temperature for 10 hours. This mixture was then distilled in vacuo. The still-residue was taken up in dry ether by slight warming, and stood at room temperature, whereby crystals began to appear. The solution containing some deposit was further chilled in an ice-salt mixture at −10° so as to complete crystallization. The product after recrystallization from dry ether weighed 0.41 g. It sintered at 75° and melted at 91°. N 3.02%, P 3.18% (Calc. for $C_{50}H_{89}O_{13}N_2P$: N 2.92%, P 3.24%).

Cleavage of phthalyl group. 0.40 g. of the phthalylglutamylcephalin was dissolved in 12 cc. of ethylcellosolve and 0.03 cc. of 90% hydrazine hydrate was added in drops under agitation. The mixture was heated for 30 minutes under reflux in a boiling water bath, whereby it became markedly cloudy, and cooled. Thereafter it was neutralized with 1 *N* HCl, poured into 100 cc. of ice-water, and without separating off the flocculent precipitate, exhausted with changes of chloroform. The combined chloroform solutions were washed well with water, dried with $CaCl_2$ and distilled. The remainder was dissolved in the smallest possible volume of an acetone-ether (1 :1 by volume) mixture and stood overnight in an ice chest. A half-crystalline deposit given was centrifuged and dried in a desiccator ($CaCl_2$). Yield 0.35 g. It contracted at 135° and decomposed gradually on further raising of the temperature.

Condensation with N-acetylglucosamine-4-β-galactoside. In a manner similar to above 150 mg. of the γ-glutamyl-cephalin and 65 mg. of the disaccharide were condensed. The purified product, which looked like crystals and showed birefringence in polarized light, amounted to 35 mg. Molisch strongly positive. The indirect-Osaki-Turumi reaction positive, but not the direct. N 3.27%, P 2.50%, glucosamine 15.09% (Calc. for $C_{55}H_{104}O_{21}N_3P$: N 3.58%, P 2.81%, glucosamine 15.26%). It contracted with slight browning at 82–85° and decomposed at 187°.

7) Sphingosine-N-α-(N-acetyl)-glucosaminido-4-β-galactosido-2-β-(N-acetyl)-glucosaminido-4-β-galactoside (**VI**)

Sphingosine sulfate was prepared according to Klenk & Diebold[26] from human brain. It broke up under browning at 235–245°. Free sphingosine was derived from the sulfate, and 20 mg. of it and 100 mg. of the tetrasaccharide were

boiled with 2 cc. of absolute methanol under a return condensor (ground joint), avoiding moisture, in a current of dry CO_2 gas for 12 hours (glycerol bath) and then allowed to cool in air. The precipitate was centrifuged off. The supernatant was distilled in vacuo to a syrup, washed with a large excess of dry ether and dried in vacuo over $CaCl_2$. It was next taken up in 5 cc. of warm chloroform, some insoluble was eliminated by rapid filtration while warm, and the filtrate was condensed to a thick syrup as above, washed with 5 cc. of dry ether and dried. This was further purified as follows:— It was taken up in the smallest possible quantity of warm absolute methanol (1 cc.). A small insoluble part appearing on cooling was discarded after centrifugation and the supernatant was precipitated by addition of 5 cc. of dry ether and centrifuged. The centrifugate here was dissolved in 1 cc. of absolute methanol, and after cautious addition of dry ether to persistent turbidity, placed in an ice chest. Crystal-like granules came out, which were centrifuged, washed with dry ether and dried in vacuo over $CaCl_2$. Yield 42 mg. The product turned light brown at about 80°, then contracted more and more and broke up at 180–190°. It was ninhydrin negative, but became ninhydrin positive when it was warmed (50°, 5 hours) in advance with 0.2 *N* acetic acid. Fehling reaction occurred, though very slowly. Molisch test resulted in strong positive. N 4.09%, glucosamine 32.5% (Calc. for $C_{46}H_{82}O_{22}N_3$: N 4.18%, glucosamine 34.8%).

8) Sphingosine-N-*α*-(N-acetyl)-glucosaminido-4-*β*-galactoside (**VII**)

This N-glycoside was prepared similar to above by heating the components together in absolute ethanol for 5 hours. In the subsequent fractionation, cold chloroform and absolute ethanol were employed instead of hot chloroform and absolute methanol. Starting with 50 mg. of sphingosine and 80 mg. of the disaccharide, 35 mg. of the product were given. It turned brown at about 115° and decomposed at 130°. N 4.18%, glucosamine 25.5% (Calc. for $C_{32}H_{59}O_{12}N_2$: N 4.22%, glucosamine 26.9%).

9) *α*-Sphingosine (Niemann)-N-*α*-(N-acetyl)-glucosaminido-4-*β*-galactoside (**VIII**)

From 2 g. of sphingosine sulfate prepared according to Klenk & Diebold were obtained 1.4 and 0.4 g. respectively of the *α* and *β* isomers (sulfates) after Niemann[27]. Each of them was converted into free base at 0°C by the routine procedure and immediately condensed with the disaccharide by boiling with absolute ethanol for 2 hours. The condensation product with 60 mg. of *α*-sphingosine and 90 mg. of the disaccharide amounted to 30 mg. N 4.20% (Calc. 4.22%).

10) 1-O-Palmitylsphingosine-N-*α*-(N-acetyl)-glucosaminido-4-*β*-galactoside (**IX**)

N-Anisal(p-methoxybenzylidene)-sphingosine. 650 mg. of free sphingosine were dissolved in 4 cc. of ethanol and shaken well with 440 mg. of anisaldehyde. Water was added drop by drop under vigorous stirring till appearance of faint persistent turbidity (12 cc. of water were requested). The mixture was chilled at about −10° in an ice-salt mixture, scratching the vessel-wall frequently. Fine oily droplets occurred and turned soon into colorless needles. They were

filtered cold, washed with an ice-cold mixture of alcohol and water (1 : 4 by volume) and dried in vacuo over $CaCl_2$. Yield 0.725 g. The product melted at 40° with charring. N 3.18% (Calc. for $C_{28}H_{44}O_3N$ 3.33%). It became tinted orange on standing even in a vacuum-desiccator containing $CaCl_2$.

1-*O*-*Palmityl*-(*N*-*anisal*)-*sphingosine*. 700 mg. of a fresh preparation of the compound above were dissolved in a mixture of 5 cc. of dry, alcohol-free chloroform and 1 cc. of dry quinoline, and a solution of the equivalent amount (630 mg.) of palmitylchloride in 3 cc. of the chloroform were added in small portions in the course of 20 minutes, under agitation. The reaction mixture was stood for 2 hours at room temperature (5°), washed three times with watery 1 *N* HCl and four times with water and made clear by means of anhydrous calcium chloride, followed by vacuum distillation to thorough dryness. The remainder partially changed into crystals on addition of 3 cc. of dry ether. This mixture was stood at −10° in order to complete the change. The crystals with yellow hue thus given were filtered off, and after washing once with cold ether, dried in vacuo over $CaCl_2$. Yield 210 mg. The product melted at 68° under charring. N 1.95 % (Calc. for $C_{42}H_{73}O_6N$ 1.98%).

1-*O*-*Palmitylsphingosine hydrochloride* (*Cleavage of anisal group*). 180 mg. of 1-O-palmityl-(N-anisal)-sphingosine were suspended in 6 cc. of an acetone-ether (1 : 1 by volume) mixture and after addition of 0.15 cc. of watery 2 *N* HCl, boiled for a few minutes until a perfectly transparent solution was given. Thereafter the solution was cooled at −10°. Long non-colored prisms that precipitated were quickly centrifuged (30 sec. at 3000 r.p.m.), washed with ice-cooled ether and dried in vacuo over H_2SO_4—Cryst. I. The yellowish mother fluid of Cryst. I was distilled in vacuo to dryness, taken up in 2 cc. of ether and chilled at −10°. A deposit of crystals resembling the ones above but with yellowish tint was obtained—Cryst. II. Yield : Cryst. I 48 mg.; Cryst. II 10 mg. F.P. : Cryst. I 185° (decomp.) ; Cryst. II 182° (decomp.). N : Cryst. I 2.30% (Calc. for $C_{34}H_{67}O_3N{\cdot}HCl$ 2.44%).

1-*O*-*Palmitylsphingosine*-*N*-*α*-(*N*-*acetyl*)-*glucosaminido*-4-*β*-*galactoside*. 1-O-Palmitylsphingosine hydrochloride weighing 40 mg. was dissolved in 4 cc. of absolute ethanol, and shaken for 1 hour with a small amount of fine silver oxide powder to free the base. Filtered. The filtrate was added to with 70 mg. of the disaccharide and 0.015 cc. of glacial acetic acid and boiled under reflux (glycerol bath) for 5 hours. The condensation product was isolated as in 7) and reprecipitated from ethanol by the aid of ether, and 24 mg. of a white amorphous substance were yielded. Ninhydrin negative; positive Fehling reaction occurred but very slowly ; Molisch strongly positive. Soluble in ethanol, methanol and chloroform ; insoluble in ether and acetone. N 3.50%, glucosamine 21.92% (Calc. for $C_{48}H_{89}O_{13}N_2$: N 3.62%, glucosamine 22.3%).

Group Potencies of Compounds I–IX

Method of assay

0.4 cc. of neutral 1% solutions or emulsions of the substances in physiological saline were progressively-doubled soon after preparation with the saline

in a range of test tubes, taking 0.2 cc. from the preceding tubes, and immediately thereupon 0.2 cc. of the sera, which had been diluted to titre 16, were added. The mixtures were stood at 37°C for 2.5 hours with occasional shaking at first and afterwards at 0–5°C for 4 hours. The subsequent hemagglutination stage was processed in test tubes excepting for the compounds **VI–IX**. Namely, the mixtures were added to with 2 drops of a 5% erythrocyte suspension and placed in a water-thermostat at 37°C. Controls were carried out in parralel, by standing in the thermostat the sera, once diluted to titre 16 as above, after further progressive dilution and addition of erythrocytes. The group potencies of the specimens were judged by determining by means of a magnifier their dilutions wehre they inhibited hemagglutination, just when the sera diluted to titre 1, i. e., those at a dilution of 1 : 16 of the sera once diluted to titre 16, in the control runs began the agglutination. The corresponding stage regarding **VI–IX** was made on a glass plate, employing 1 drop of the absorbed sera and 1 drop of erythrocyte suspensions. The mixtures here were maintained at room temperature, and the results were judged under conditions similar to those above.

Results

The results of the assay are illustrated in Table I.

Relationship between Mutarotation and Group Specificity

1) On one hand, 15.0 mg. of Compound **VII** were dissolved in 0.75 cc. of absolute ethanol and filled up to 1.5 cc. by addition in portions of *M*/15 phosphate buffer pH 7.0 (Sörensen). A tiny precipitate (phosphate) was quickly centrifuged off (4000 r.p.m.), and the supernatant was examined at 15°C regarding the rotation change with time. On the other hand, a 1% solution of the compound in *M*/30 phosphate buffer pH 6.8 was prepared and stood at 15°C for a series of assays of group potency at intervals (plate method for the hemagglutination test). One and the same solvent was not used unavoidably for the both examinations, because a clear solution which allows measurement of optical rotation can not be given by using the aqueous phosphate buffer alone whereas ethanol interferes with the serological assay. The results are shown in Table II and Fig. 2. Both the dextrorotation and Group A potency decreased quickly in parallel after dissolution of the N-glycoside, the former solution becoming levorotatory and the latter Group A as well as B potent, followed by a repeated reversal of rotation due to hydrolysis of the substance (The free sugar is dextrorotatory) and diminution of both Group A and B potencies.

2) Next, 1% solutions of Compound **IV** were prepared and examined as above, replacing a mixture of pyridine and *M*/15 phosphate buffer pH 7.0 (1 : 1 by volume) for the alcohol-phosphate buffer mixture to prepare the solution for optical measurement (Table II, Fig. 3). Here neither the rotation nor the group activity changed noticeably.

The findings reveal that the Group A active synthetical compounds are α-N-glycosides and the Group B active β-N-glycosides (Cf. Tsuiki[28])[29]), and further suggest that, in natural Group A and B substances, the carbohydrate

Table I

Anti-hemagglutinative Potency of Synthetical Compounds

h: hemolysis. −: no agglutination, ±: faint aggl., +: slight aggl., ++: marked aggl., +++: strong aggl.

Compound	Serum	Group of red cells	Dilution of the substances (1:)								Dilution of the sera of titre 16 in control runs (1:)				
			10^2	2×10^2	4×10^2	8×10^2	1.6×10^3	3.2×10^3	6.4×10^3	1.28×10^4	2	4	8	16	32
I	α-	A	−	−	−	+	+	+	++	++	+++	++	+	±	−
	β-	B	−	−	−	−	−	−	±	+	+++	++	+	±	−
	Eel-	O	+	+	++	++	++	++	+++	+++	+++	++	++	+	−
II	α-	A	±	±	+	+	+	++	++	++	+++	++	+	±	−
	β-	B	−	−	−	−	−	−	+	++	+++	++	+	+	−
	Eel-	O	+	+	+	+	++	++	++	++	+++	++	+	+	−
	Goat-	O	±	±	+	+	++	++	++	++	+++	++	+	±	−
III	α-	A	±	±	+	+	+	+	+		+++	++	+	±	−
	β-	B	−	−	−	−	−	±	+		++	+	+	±	−
	Eel-	O	+	+	++	++	++	++	++		+++	++	+	+	±
	Goat-	O	±	±	+	++	++	++	++		+++	++	+	±	−
	Anti-O saliva chicken-	O	±	±	+	++	++	++	++		+++	++	+	±	−
IV	α-	A	−	±	+	+	+	+	+	++	++	+	+	±	−
	β-	B	−	−	−	−	−	−	+	++	++	+	+	+	−
	Eel-	O	+	++	++	++	++	++	++	++	++	+	+	+	−
	Goat-	O	±	+	+	++	++	++	++	++	++	+	+	+	−
V	α-	A	−	±	+	+	+	+	+		++	+	+	+	−
	β-	B	−	−	−	−	−	±	+		++	+	+	±	−
	Eel-	O	+	+	++	++	++	++	++		++	+	+	+	−
	Goat-	O	±	+	+	++	++	++	++		++	+	+	+	−
VI	α-	A	h−	h−	−	−	−	−	+		++	+	+	±	−
	β-	B	h−	h±	±	+	+	+	++		++	+	+	±	−
	Eel-	O	h+	h+	++	++	++	++	++		+++	++	+	+	−
	Goat-	O	h+	h+	+	+	++	++	++		+++	++	+	+	−
	Anti-O saliva chicken-	O	h±	h+	+	+	++	++	++		+++	++	+	+	−
VII	α-	A	h−	−	−	−	−	±	+		++	+	+	±	−
	β-	B	h−	±	+	+	+	+	++		++	+	±	±	−
	Eel-	O	h+	h+	++	++	++	+++	+++		+++	++	+	+	−
	Goat-	O	h±	+	+	++	++	++	++		+++	++	+	+	−
	Anti-O saliva chicken-	O	h±	h+	++	++	++	++	+++		+++	++	+	+	−
VIII	α-	A	h−	−	−	−	−	±	+		++	+	±	±	−
	β-	B	h−	±	±	+	+	+	++		++	+	+	±	−
	Goat-	O	h±	±	+	++	++	++	++		++	++	+	±	−
	Anti-O saliva chicken-	O	h±	+	++	++	++	++	++		++	++	+	±	−
IX	α-	A	h−	−	−	−	−	±	+		++	+	+	±	−
	β-	B	h±	h±	+	+	+	+	+		++	+	+	±	−
	Eel-	O	h+	h+	++	++	++	++	++		++	++	+	+	−
	Goat-	O	h±	h+	+	++	++	++	++		++	++	+	+	−

TABLE II

Changes of Group Potency and of $[\alpha]_D$ with Time of Compounds VII (Sphingosine-N-α-(N-acetyl)-glucosaminido-4-β-galactoside) and IV (α-Glutamyl-(α,α'-dipalmitylglycero-β-phosphoryl)-ethanolamine-N-β-(N-acetyl)-glucosaminido-4-β-galactoside)

h, $-\sim+\!+$: signify the same as in Table I.

Time of standing (hrs.)	Assay of group specificity: Serum used	Group of red cells	Dilution of the substances (1 :) 10^2	2×10^2	4×10^2	8×10^2	1.6 $\times10^3$	3.2 $\times10^3$	6.4 $\times10^3$	Dilution of the sera of titre 16 in control runs (1 :) 2	4	8	16	$[\alpha]_D$ (°)
A. Compound VII														
1/4	α	A	h−	−	−	−	−	±	++	++	+	+	±	+17.5
	β	B	h−	−	±	+	+	++	++	++	+	+	±	
1	α	A	h−	−	−	−	+	+	++	++	+	+	±	−2.0
	β	B	h−	−	−	±	+	++	++	++	+	+	±	
2	α	A	h−	−	−	+	+	+	++	++	++	+	±	−6.5
	β	B	h−	−	−	−	±	+	++	++	++	+	±	
3														−10.0
4														−10.0
5	α	A	h−	±	±	+	+	++	++	++	+	±	±	−7.5
	β	B	h−	−	±	±	+	++	++	++	+	±	±	
10														0.0
12	α	A	h±	±	+	+	++	++	++	++	+	±	±	+2.5
	β	B	h−	±	+	+	++	++	++	++	+	±	±	
15														+3.5
24														+3.0
B. Compound IV														
immediately after dissolution	α	A	±	+	+	+	++	++	++	++	+	±	±	+4.5
	β	B	−	−	−	−	−	−	+	++	+	+	±	
1	α	A	±	±	+	+	++	++	++	++	+	+	±	+4.0
	β	B	−	−	−	−	−	−	+	++	+	+	±	
2	α	A	±	±	+	+	++	++	++	++	+	+	±	+5.5
	β	B	−	−	−	−	−	−	+	++	+	±	±	
3														+5.0
5														+6.3
12	α	A	+	+	+	+	++	++	++	++	+	+	±	+6.0
	β	B	−	−	−	−	−	−	+	++	+	+	±	
24														+6.0

moiety is linked to the aglycon moiety of non-carbohydrate nature α- and β-glycosidically in general.

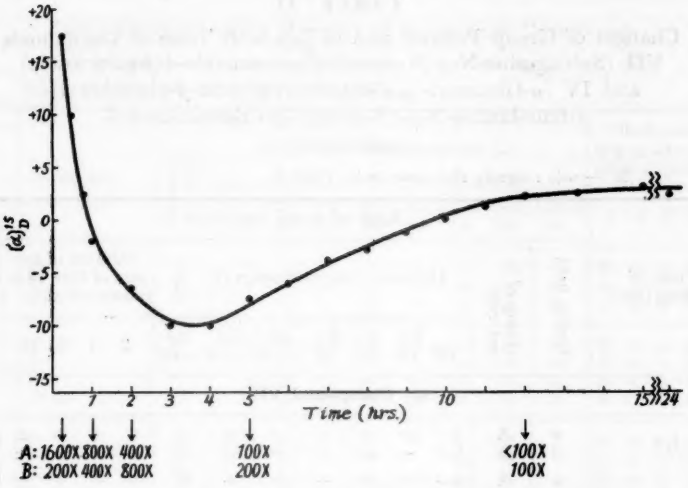

Fig. 2. Mutarotation and change of group activity with time of Compound **VII** in solution (Regarding details, see the text and Table II).

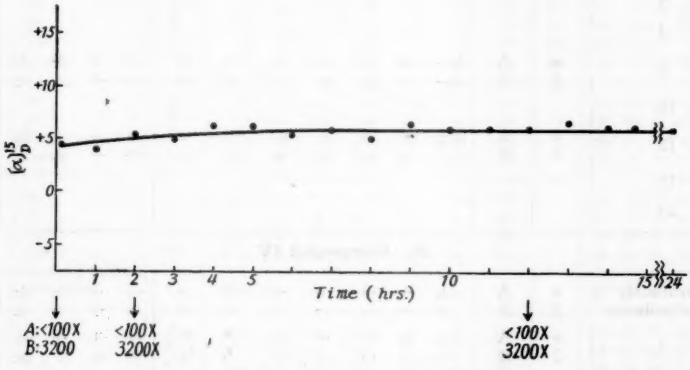

Fig. 3. Behaviours of optical rotation and group activity of Compound **IV** stood in solution (Regarding details, see the text and Table II).

Other Compounds Synthesized and Serologically Assayed

Besides the compounds above, we have synthesized not a few N-glycosides, some of which exhibited group activity though not typical. Their names will be enumerated, appending group active dilutions in parentheses with respect to the group active: phosphatidylserine-N-(N-acetyl)-glucosaminido-4-β-galactoside (A $1:10^2$, B $1:4\times10^2$), α-D-glutamyl-(α,α'-dioleylglycerophosphoryl)-ethanolamine-N-(N-acetyl)-glucosaminido-4-β-galactosido-2-β-(N-acetyl)-glucosaminido-

4-β-galactoside (A $1:8\times10^2$, B $1:3.2\times10^3$), α-D-glutamyl-(α,α'-dioleylglycerophosphoryl)-ethanolamine-N-(N-acetyl)-glucosaminido-4-β-galactoside (A $1:4\times10^2$, B $1:3.2\times10^3$), sphinogosine-N-L-fucoside (E $1:4\times10^2$), sphingosine-N-galactoside, sphingosine-N-(N-acetyl)-glucosaminide (A $1:4\times10^2$, B $1:10^2$), sphingosine-N-lactoside, β-sphingosine(Niemann)[27]-N-(N-acetyl)-glucosaminido-4-β-galactoside (A $1:4\times10^2$, B $1:2\times10^2$), dihydrosphingosine-N-(N-acetyl)-glucosaminido-4-β-galactoside (A $1:4\times10^2$, B $1:4\times10^2$, O (goat serum) $1:10^2$, O (anti-O saliva chicken-serum) $1:10^2$), mugirin(Hirohata)-N-(N-acetyl)-glucosaminido-4-β-galactoside, zein-N-(N-acetyl)-glucosaminido-4-β-galactoside, α-glutamylglutamic diethylester-N-(N-acetyl)-glucosaminido-4-β-galactoside (E $1:10^2$), glutamic diethylester-N-lactoside.

Summary

1. To verify our hypothesis regarding the configuration requisite for developing A and B group specificity, we have prepared a number of N-glycosides, of which the following proved typically group active: phosphatidyl-(α-glutamyl)-ethanolamine-N-β-(N-acetyl)-glucosaminido-4-β-galactosido-2-β-(N-acetyl)-glucosaminido-4-β-galactoside (**I**), 2) hydrophosphatidyl-(γ-carbomethoxy-α-aminobutyryl-glycyl-glycyl)-ethanolamine-N-β-(N-acetyl)-glucosaminido-4-β-galactosido-2-β-(N-acetyl)-glucosaminido-4-β-galactoside (**II**), hydrophosphatidyl-(γ-carbomethoxy-α-aminobutyryl-glycyl-glycyl)-ethanolamine-N-β-(N-acetyl)-glucosaminido-4-β-galactoside (**III**), α-glutamyl-(α,α'-dipalmitylglycero-β-phosphoryl)-ethanolamine-N-β-(N-acetyl)-glucosaminido-4-β-galactoside (**IV**), γ-glutamyl-(α,α'-dipalmitylglycero-β-phosphoryl)-ethanolamine-N-β-(N-acetyl)-glucosaminido-4-β-galactoside (**V**), sphingosine-N-α-(N-acetyl)-glucosaminido-4-β-galactosido-2-β-(N-acetyl)-glucosaminido-4-β-galactoside (**VI**), sphingosine-N-α-(N-acetyl)-glucosaminido-4-β-galactoside (**VII**), α-sphingosine (Niemann)-N-α-(N-acetyl)-glucosaminido-4-β-galactoside (**VIII**), 1-O-palmitylsphingosine-N-α-(N-acetyl)-glucosaminido-4-β-galactoside (**IX**).

2. **I–V** were Group B active at dilutions of $1:3.2\times10^3$, $1:3.2\times10^3$, $1:1.6\times10^3$, $1:3.2\times10^3$ and $1:1.6\times10^3$ respectively and **VI–IX** Group A active at dilutions of $1:3.2\times10^3$, $1:1.6\times10^3$, $1:1.6\times10^3$ and $1:1.6\times10^3$ respectively.

3. Behaviours of **VII** and **IV** examined regarding the relationship between optical rotation and group potency showed that Group A synthetical substances are α-N-glycosides and Group B substances β-N-glycosides, suggesting in turn that in natural Group A and B substances the carbohydrate moiety is bound to the non-carbohydrate moiety α- and β-glycosidically respectively in the molecule.

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which is gratefully acknowledged. H. Masamune.

References and Notes

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2) Masamune, Tohoku J. Exp. Med., 1953, **57**, 310.
2a) Masamune, Ogawa & Akama, *ibid.*, 1953, **58**, 234.
3) Masamune, Hakomori, Maehara & Suzuki, *ibid.*, 1954, **59**, 231.
4) Hirata, *ibid.*, 1954, **59**, 247.
4a) Hakomori, *ibid.*, 1954, **60**, 331.
5) Yamakawa & Iida, Jap. J. Exp. Med., 1953, **23**, 327.
6) Yamakawa & Suzuki, J. Biochem., 1952, **39**, 393.
7) Cf. Masamune & Yosizawa, Tohoku J. Exp. Med., 1954, **60**, 135.
7a) Yosizawa, *ibid.*, 1949, **51**, 51.
8) Folch, J. Biol. Chem., 1942, **146**, 35.
9) Alcohol free. Commercial chloroform was washed with a dilute NaOH, a dilute HCl and water in turn, dried with $CaCl_2$ and further with P_2O_5 and distilled.
10) Prepared according to Bergmann & Zervas (Ber. deut. chem. Ges., 1932, **65**, 1192).
11) The ninhydrin reagent used in the present work was Stein & Moore's modified by Masamune, Hakomori & Masamune (Tohoku J. Exp. Med., 1954, **60**, 347). By this reagent, not only the purified natural phosphatidylethanolamine but also synthetical dipalmitylglycerophosphorylethanolamine (See 5) in the same chapter) exhibited strong positive reaction, although colaminecephalins have been considered inert in this respect.
12) Willstätter & Waldschmidt-Leitz, Ber. deut. chem. Ges., 1921, **54**, 123.
13) Much larger than the theoretical volume. When phosphatidylethanolamine itself was treated in a similar manner, the consumption of hydrogen reached only 1/3 of the calculated for 1 double bond.
14) Very active pyrophoric Raney Ni was prepared by digesting the Ni-Al-alloy according to Billica & Adkins (Cope & Nace, Org. Syntheses, Vol. 29, p. 24). Levene & West, Salisbury & Anderson and Gray hydrogenated cephalin and lecithin using nobel metal catalysts. However, these catalysts are often poisoned by a trace of contaminant of the phosphatides in contrast to the active Raney Ni.
15) Yamashita & Yashiro, Nippon Nogeikagaku Kaishi (J. Agr. Chem. Soc. Jap.), 1954, **28**, 674.
16) Purified according to the description of Fieser (Experiments in Organic Chemistry, 2nd ed., Reinhold Publ. Corp. New York, 1942, p. 381).
17) Kindly furnished from the Organic Chemical Laboratory of Prof. Nozoe, Faculty of Science. It was converted into diazomethane by the method described in Backmann & Struve, Organic Reaction, Vol. 1, 1935, p. 50, and Arndt, Organic Syntheses, Vol. 15, 1935, p. 4.
18) Methyl, which was attached to the phosphoryl residue when the compound was treated with diazomethane, was proved by alkaline titration to have been cleft off during decarbobenzoxylation.
19) Fresh preparation after distillation avoiding moisture.
20) Rose, J. Amer. Chem. Soc., 1947, **69**, 1384.
21) Ethanolamine hydrochloride, generously supplied by Dr. S. Takizawa in the Applied Chemistry Department, Faculty of Technology, was changed into free base by dissolving 10 g. of it in a calculated volume of 20% NaOH followed by distillation. The distillate was dropped alternatively with 15% NaOH (27 cc. in total) onto the calculated amount of carbobenzoxychloride (17 g.) during 1 hour, under vigorous stirring and ice-cooling, and the mixture was further shaken at room temperature for 30 minutes. A lot of needles which came out were

separated by filtration and recrystallized from ether. F.P. 62°.

22) Bear *et al.* (J. Amer. Chem. Soc., 1952, **14**, 154) succeeded to cleave off the carbobenzoxy group from carbobenzoxycephalin by hydrogenolysis, contrary to Rose (20)), who claimed it impossible and recommended reduction with phosphonium iodide according to Harington & Mead.

23) Tausz & Putnocky, Ber. deut. chem. Ges., 1919, **52**, 1573.

24) King, Jackson & Kidd, J. Chem. Soc., 1951, 243.

25) Kidd & King, J. Chem. Soc., 1949, 3315.

26) Klenk & Diebold, Z. physiol. Chem., 1931, **198**, 25.

27) Niemann, J. Amer. Chem. Soc., 1941, **63**, 1763. Niemann pronounced that his α- and β-sphingosine take cis and trans configurations respectively, being based on their difference in hydrogen absorption and fluorescence. Our results that a Group A substance was produced with α-sphingosine like with natural sphingosine and the scarcely group-active with β-sphingosine suggest the natural sphingosine to be the α-isomer mostly at least whether it is cis or trans form (Cf. Mislow, J. Amer. Chem. Soc., 1952, **74**, 5155).

28) Tsuiki, Tohoku J. Exp. Med., this volume, p. 267.

29) The N-glycosidic form α or β has been assigned to the synthetical compounds **I–IX** on this basis.

Note at proof-reading : This article has been read before the 26th Annual Meeting of the Japanese Biochemical Society, Sendai, April 25～27, 1954.